

Biographical Article

Mary Shaw Shorb (1907–1990)

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Mary Shaw was born on January 7, 1907, in Wahpeton, North Dakota, on the Red River just across the border from Minnesota. Her parents were Mary McKean and Ernest Shaw.

Ernest Shaw's father, Alexander Perigo Shaw, emigrated from the Isle of Skye, Scotland, before the American Civil War and served in the Union Army. Mary McKean was of Scottish-Irish-English ancestry and could trace her lineage back to four of the original colonists at Plymouth Rock: John Alden, Priscilla Mullens, Governor William Bradford and Mrs. Bradford. Mary Shaw's grandfather, Charles Arthur McKean, also served in the Union Army during the American Civil War.

Ernest Shaw was born in 1873 in Michigan and made his living as an abstractor, that is, one who describes parcels of property for plot maps. Mary McKean was born in 1871 in Minnesota and made her living as a school teacher. They met in North Dakota and were married in Wahpeton in 1904. Mary Shaw was the second of their five children.

When Mary was three years old the family moved to Caldwell, Idaho. All five Shaw children attended

Caldwell public schools and the College of Idaho, also in Caldwell. Ernest Shaw spent some time as president of the Caldwell School Board.

A neighbor and family friend, William Judson Boone was the founder and president of the College of Idaho. A botanist whose specialty was taxonomy, President Boone collected wildflowers for classification and was recognized throughout Caldwell as the person who knew the edible mushrooms. Mary Shaw was impressed at an early age by Dr. Boone's knowledge. It made biology a particularly relevant field of study to her.

Mary met Doys Shorb, her future husband, at kindergarten classes shortly after the Shaw family moved to Caldwell. In their teenage years they often hunted mushrooms together and went on fishing trips with Dr. Boone where they provided the mushrooms, he caught the fish, and they all searched for unusual wildflowers. Mary was very interested in wildflowers wherever she traveled all of her life.

Her early interest in biology was reinforced when Mary Shaw entered the College of Idaho in 1924. Two faculty members inspired her career as a nutrition scientist: Orma J. Smith, a zoologist, who convinced her to major in biology and Margaret F. Nichol, a home economist, who led her to minor in that field. Mary Shaw was an outstanding student at the College of Idaho, serving as director of Founders Day and editor of *The Trail*, the college yearbook, in 1928. She earned a B.S. degree in biology with a minor in home economics that year.

Although just 4 feet 11 inches tall, Mary had a take charge personality that propelled her into leadership roles. People had little difficulty recognizing that they were dealing with a bright and capable person. She rationed words and was never effusive; a few words from Mary often had a lot of meaning.

Mary Shaw's first job was as a dietitian at Johns Hopkins University Hospital in Baltimore, selected because her older brother, Manley, was in medical school there. Mary was assigned to learn the dietetics profession from the ground up. She spent long grueling hours cooking food, filling plates and washing dishes for a solid month, but the glamour of dietetics quickly wore off and Mary began to look for a way

out. The School of Hygiene had just received a \$5,000,000 grant to study the common cold and find a cure for it. The Department of Immunology was hiring.

Mary Shaw took her résumé to immunology and was quickly hired. The project employed a large number of people, but no cure for the common cold resulted, and the money soon ran out. What did result was Mary's decision to study for the Sc.D. in immunology.

In 1929 Mary Shaw returned briefly to Caldwell to marry her childhood sweetheart, Doys Shorb. It was a marriage that was to last 61 years, ending with Mary's death. The newly married couple journeyed to Baltimore for Mary to continue her graduate studies in immunology and for Doys to enter the graduate program in parasitology.

Mary Shorb received her Sc.D. in immunology from Johns Hopkins University in 1933. Her dissertation research used rabbits to develop a heterophile antigen that proved to be effective in preventing or treating a number of human diseases, including pneumonia. Until the development of sulfa drugs, her antigen was a widely used treatment for pneumonia.

In 1933, during the depths of the Great Depression, multimillion grants to study the common cold were, at least temporarily, a thing of the past and researchers were almost unemployable, particularly female ones. Mary was turned down for a position that she was well suited to fill at Washington State College because she was a woman. The position would have allowed Doys and Mary Shorb to be closer to their families in Idaho.

Mary finally obtained a position as a social worker with the Baltimore Emergency Relief Agency. This was one of many government positions that became available through the New Deal efforts of President Franklin Roosevelt to pull the United States out of the depression.

Mary Shorb worked for the agency until, after the birth of her first child, Barbara, in 1936, she decided to concentrate on being a housewife and mother. A son, Alan, was born in 1938. During World War II, however, many male employees in all professions were in the armed forces, so there were vacancies, including research positions. In 1942, Mary gave birth to her third child, Carole Elizabeth (now called Betsy), and hesitated to reenter the work force. Her ambivalence was ended when a former Johns Hopkins University classmate told her that it was her patriotic duty to do so. So Mary took a position with the Bureau of Home Economics and Human Nutrition of the USDA at Beltsville that year.

She was one of four women scientists the USDA assigned to find a substitute for the rubber rings used in home canning; all the available rubber was being used for the war effort. Her job as a bacteriologist was to check the canned products for the presence of botulinum toxin that might be formed if the sub-

stitute rings failed. She also did nutritional studies with rats that involved homogenizing the carcasses in order to determine nutritional status. She detested the grinding of rat carcasses. As she had done in 1928, Mary looked for a way out.

She found it in the Bureau of Dairy Industry of USDA at Beltsville in 1944. They were using *Lactobacillus lactis* Dorner (LLD) to make yogurt and other fermented dairy products. Mary transferred to that Bureau for the job of culturing the microorganisms. The culture medium used tomato juice and liver extract. Nobody knew why the microorganism required the liver extract, but Mary wanted to know.

Pernicious anemia seriously afflicted 50,000 Americans yearly through 1925. Mary Shorb's father-in-law, Cormac Joseph Shorb, eventually died of this disease. In 1926 raw liver was found to be an effective—albeit distasteful—treatment for pernicious anemia. George R. Minot and William P. Murphy shared the 1934 Nobel Prize in medicine for this discovery. However, the amount of raw liver needed for therapeutic effectiveness was nearly one pound daily and advanced cases of this disease did not respond.

After this discovery, several pharmaceutical firms embarked upon the purification, isolation, and identification of the anti-pernicious anemia factor in liver. These efforts were slowed by lack of sufficient subjects for bioassays. Nevertheless a team of scientists at Merck & Company, headed by Karl A. Folkers, had cooperated with Randolph West at Columbia University, College of Physicians and Surgeons, and had been able to separate liver extracts into active and inactive fractions.

When World War II ended in 1945, Mary Shorb, along with most other married female scientists, was bumped from her position at USDA in 1946 by the returning veteran who held it previously. If they had any interest in her idea that LLD could be a useful assay tool for human nutrition, the Bureau of Dairy Industry at USDA could have retained her, but they had no such interest. Mary was without a laboratory at a time when she felt able to contribute to the research effort against pernicious anemia.

At that point a window of opportunity opened. George Briggs had joined the faculty of the Poultry Husbandry Department of the nearby University of Maryland before moving on with his own distinguished career in nutrition. While there he spoke to his department head, Morley Jull, about Mary's predicament and got her an offer of laboratory space.

Mary was given official leave without pay status from the University of Maryland on August 1, 1946. This gave her a base from which to solicit research funding, but her first efforts were unsuccessful. A number of major pharmaceutical firms saw little merit in Mary's hypothesis that the LLD bacterium could be used as a rapid biological assay for the anti-pernicious anemia factor, even though microbiological assays were used successfully in the isolation

of folic acid. Indeed, a group at the Merck laboratories (Keresztesy et al. 1943) used a microbiological assay to isolate a derivative of folic acid (rhizopterin).

Immediately following one of these rejections, Karl Folkers of Merck traveled from Rahway, New Jersey, to College Park, to speak with George Briggs about possible collaborative research. As he was putting on his coat to leave, he asked Dr. Briggs what the University of Maryland would expect from Merck and Company in such collaboration. In response, Dr. Briggs pulled out a copy of Mary's rejected research proposal from his desk.

At that point, Karl Folkers saw his own window of opportunity open. He went back to Rahway and worked to convince his superiors of the potential merit of this proposal. He finally decided to request an initial \$400. A larger initial request would have probably been refused, but this modest amount was not.

What Karl Folkers wanted for this initial investment was for Mary to assay a set of liver extract samples that were unknown to her. Some of these samples were known by Randolph West, through his work with human pernicious anemia patients, to be rich in the anti-pernicious anemia factor and some were intentionally lacking this factor. Mary unerringly identified the samples active in human pernicious anemia patients in a matter of hours. Merck and Company was convinced. Larger sums were almost immediately appropriated, and these sums kept coming for the rest of Mary's professional life.

During 1947 Mary collaborated with the research team of Karl Folkers at Merck and with Randolph West at Columbia. The Folkers team sent her fractions of liver extract and she used LLD to tell them how much of the anti-pernicious anemia factor each contained. The LLD bioassay was rapid and the collaboration led the Merck team to isolate the substance that they named vitamin B-12 in pure crystalline form from both a fermentation source and from liver within about three months.

In retrospect, the color of vitamin B-12 and the ease of its crystallization facilitated isolation and removed the burden from the LLD assay. Edward L. Rickes at Merck originally used column chromatography to examine fermentation sources of the anti-pernicious anemia factor and found a pink band on his column, and he asked Dr. Folkers what he thought that pink material might be. Dr. Folkers replied that Rickes should allow the pink band to pass down the column and be separated for study. Red crystals were very readily obtained that proved to be active in the LLD bioassay and were actually vitamin B-12.

The testing of these red crystals in human subjects may read like a fantasy to those familiar with the legal restrictions of the 1990s. There was no prior safety testing in even one animal, but Dr. Folkers and Dr. West thought that an injection of a solution of

these red crystals obtained from previously tested liver samples would be safe for humans. Norman G. Brink at Merck and Company had extraordinary skill in micromanipulation and obtained a few hundred micrograms of the red crystals from liver.

This preparation was almost immediately tested on several virgin cases of pernicious anemia by Dr. West at Columbia and proved to be therapeutically effective. A dose of three micrograms injected into such a patient gave a complete hematological response. The results of this work were published as three successive papers in the April 16, 1948, issue of *Science* (Rickes et al. 1948, Shorb 1948, West 1948). Professional accolades were bestowed on everyone involved.

Mary Shorb and Karl Folkers were corecipients of the Mead-Johnson Award of the AIN in 1949. Mary was well known from that day forward. She was always quick to acknowledge that she owed a great debt to Karl Folkers.

In 1949 Mary Shorb was appointed a full research professor at the University of Maryland. She preferred that title because it allowed her to pursue her research interests without teaching any formal courses. She did not like to lecture. Graduate students from a number of academic departments came to work with her and the University of Maryland used her extensively in its publicity as an example of the opportunities for women at College Park.

This was important to the University of Maryland initially because its president during Mary's first eight years on the faculty was H. C. "Curley" Byrd, the former football coach. The football teams during those eight years were nationally ranked and went to many bowl games, but the University was in danger of losing its accreditation (Callcott 1966). The administration's view of women in society seemed unenlightened even for that era.

Mary became a living example of a professionally competent, successful female faculty member during the Byrd era. There were very few others.

To put Mary's achievements into historical perspective for readers who did not experience this time, college and university enrollments were exploding. The faculty had been forced to teach twelve months on a ten-month salary as a wartime sacrifice, and then, because of the crush of veteran enrollment, waited two full years after the war ended before vacation periods and salaries could be adjusted. Mary's appointment to a position requiring no teaching after such an era indicates the respect given to her research.

Mary Shorb's later research included work on the effects of antibiotics on poultry, the lipid content of swine parasites, food bacteriology and growth factors in chicks. She was the author or co-author for 58 refereed journal articles and numerous abstracts and popular press articles before her retirement in 1972. In 1970 Merck and Company gave \$10,000 to the

University of Maryland to establish a Shorb lectureship through which many leading nutrition researchers have been invited to College Park to discuss their research with faculty and graduate students. Drs. George Briggs and Karl Folkers were among the early invitees.

Mary Shaw Shorb was a child of her time. Although she was long presented to the world as a woman who made it by overcoming bias in a male-dominated profession, she preferred not to recall prejudice in some of her last public statements. She remained silent when asked to criticize male colleagues in her past. The truth was that she worked well with men and could attribute much of her professional success to her ability to do so.

We now know enough about the chemistry of vitamin B-12 (cobalamin) to realize that the LLD assay that Mary used would not have worked in all systems. The cobalamin molecule consists of a corrin nucleus, an aminopropanol, a sugar and a nucleotide. The corrin nucleus contains cobalt and is the central structure of the entire class of compounds termed corrinoids. Cobalamins are one type of corrinoid.

Corrinoid molecules are not active as vitamin B-12 in humans because they differ structurally from cobalamins (they contain different side chains attached to the corrin nucleus) and are often referred to as analogs of vitamin B-12. However, these vitamin B-12 analogs are active as vitamin B-12 in some bacteria. Bacteria such as LLD need only the corrin nucleus of the molecule for vitamin activity.

The liver and fermentation preparations that Mary Shorb analyzed for vitamin B-12 in 1947 did not contain significant amounts of vitamin B-12 analogs. Later potential sources of vitamin B-12 analyzed by LLD did have such analogs and the assay proved itself to be inaccurate. She used several serial transfers to eliminate these sources of error and continued to use the LLD bioassay in her laboratory for vitamin B-12 for many years. It was, after all, the bioassay that had brought her fame, but the procedure is no longer in use.

In addition to the 1949 Mead Johnson Award of AIN, Mary Shorb received the 1948 Hematology Research Foundation Award, the 1957 Sigma Xi Research Award, and was named Hood College's Outstanding Woman of Maryland in 1951. The College of Idaho named her as a Distinguished Alumnus in 1966, and, in 1978, it awarded her an honorary Doctorate of Science. In 1987 Mary Shorb was inducted into the Maryland Women's Hall of Fame. In 1988 she was selected as the Woman of the Year by Prince George's County and the Scientist of the Century by the Maryland Agricultural Experiment Station.

Essex Community College in Maryland did a television documentary of her life in 1989. She was asked in that documentary how she felt about all these awards late in her life. She said, "It is nice, but, it would have meant a lot more 40 years ago." Forty

years earlier there had been many who failed to give her proper recognition while praising her male colleagues on the same research project.

Mary read mystery novels for relaxation and left behind a collection of more than 500 of these books when she died, including all the novels of Agatha Christie. Her greatest pleasure came from identifying the culprit before the author revealed "who done it".

Mary Shorb loved to travel and specifically requested a ten-month contract at the University of Maryland so that she and Doys could spend the summers traveling. She celebrated her retirement in 1972 by traveling around the world with her husband, a trip that took more than a year to complete. In 1961 she had resolved that they would take at least one international trip a year together. She made this resolution because she found her first international trips to present scientific papers on her vitamin B-12 studies in 1948, 1952 and 1956 to be lonely and unsatisfying experiences without her spouse. She never traveled alone again. Mary and Doys visited 94 countries, all the Canadian provinces and territories and all 50 of the United States during their 61 years of married life. On the trips prior to Mary's retirement in 1972, presenting research results to professional meetings was often the ostensible reason for the travel.

Mary Shorb suffered a mild stroke in 1982 and required a cane, then crutches, and finally a walker before becoming entirely bedridden in 1988. In August of 1990 she was hospitalized with pneumonia and placed on a respirator. She died on August 18, 1990, from kidney failure.

Mary Shaw Shorb was 83 years old when she died. She was survived by her husband, Doys; her son, Alan; two daughters, Betsy Shorb and Barbara Breeden; her sister, Margaret; seven grandchildren, and 14 great-grandchildren. She is interred at George Washington Cemetery in Adelphi, Maryland.

In addition to her productive scientific career, Mary Shorb led various Beltsville community groups, including the first High Point High School PTA, the Beltsville Garden Club, and the Prince Georges County Mental Health Society. In 1988, two years before her death, she was asked to explain how it was all possible. She concluded that she had a supportive spouse. Doys did much of the child-rearing and shared in the household chores.

She had some thoughts about this. "The children might have felt neglected when they were younger. My work was of prime importance," she said. "I think they're proud of me." Indeed they are.

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NOTES ON THE HISTORY OF VITAMIN B-12

Richard Ahrens's excellent biographical sketch has provided valuable information on the history of vitamin B-12. The role of chance in research is shown by his description of the visit of Karl Folkers to George Briggs. With Dr. Ahrens's approval, I am adding some more information about vitamin B-12.

The name vitamin B-12 was given by Rickes et al. (1948) to the anti-pernicious anemia factor that they had isolated in crystalline form. As noted, the original observation of anti-pernicious anemia factor is accredited to Minot and Murphy, who found in 1926 that feeding large amounts of raw liver alleviated the symptoms of patients with pernicious anemia. It may well be that it was folic acid in the liver, rather than anti-pernicious anemia factor, that produced the response. The patients were lacking in intrinsic factor (see below) and therefore would have been unable to use anti-pernicious anemia factor given orally in small amounts. One pound of liver would contain about 1.5 mg of folic acid; the RDA is 0.4 mg. However, feeding of liver to pernicious anemia patients was soon replaced by injection of liver extract, low in folic acid, but containing the anti-pernicious anemia factor.

Concentration and identification of anti-pernicious anemia factor proceeded rather slowly during the years following Minot and Murphy's original finding. Tests were with human patients. For treatment, the factor was usually administered by injections of refined liver extract containing 15,000 U anti-pernicious anemia factor/L. One unit was the amount thought to be needed per day. W. B. Castle showed in 1927 that another factor, present in the lining of normal stomachs, was needed for the uptake of anti-pernicious anemia factor from the digestive tract. He called this the intrinsic factor, and the extrinsic factor was anti-pernicious anemia factor. Further interest developed in the 1940s when it was shown that chickens needed an "animal protein factor," absent from soybean meal, but present in fish meal, and produced in fermentation by intestinal microorganisms (Jukes and Stokstad 1951, Stokstad and Jukes 1949). This brought attention to microorganisms as sources of anti-pernicious anemia factor, for animal protein factor was also present in refined liver extract, and evidently was identical with anti-pernicious anemia factor. Dr. Mary Shorb made a great contribution when she found that LLD gave a rapid test for anti-pernicious anemia factor. This accelerated the progress of the Merck group in their work to isolate anti-pernicious anemia factor. Shorb described her test in 1947 (Shorb 1947). A later publication (Shorb and Briggs 1948) outlines some of the difficulties involved; the authors say that the culture dissociates after serial transfer and "erratic results may be ob-

tained with the dissociating culture or with some crude materials because of the presence of inhibiting materials." Perhaps for these reasons, the LLD test does not seem to have been widely used.

Several groups of investigators have described their difficulties with the LLD assay. Shive et al. (1948b) said that "a medium suitable for assay techniques has not been adequately described for this organism." They found that the test responded to "sterile, aerated distilled water," especially when ascorbic acid was added. Skeggs et al. (1948) cited difficulties, and Greene et al. (1949) found that maximal growth occurred when reducing agents were added. A Merck group (Koditschek et al. 1949) found that anaerobic conditions eliminated the "LLD factor requirement" and that presence of CO₂ was necessary.

Rickes has described how anti-pernicious anemia factor crystallized in a test tube while he left his laboratory desk to answer the telephone (Jukes 1977).

Anti-pernicious anemia factor was also simultaneously isolated by E. Lester Smith (1948) of Glaxo Laboratories in England. He conducted testing by using human subjects, and he used its red color to follow the course of concentration. He also identified cobalt as a component of the molecule of anti-pernicious anemia factor (Smith 1949).

In 1948, our group at Lederle described a response by a test microorganism, *Lactobacillus leichmannii* 313, to the crystalline anti-pernicious anemia factor, which we obtained from Glaxo Laboratories (Hoffmann et al. 1948).

Dr. Mary Shorb is the discoverer of the *Lactobacillus lactis* Dorner test, rather than the discoverer of vitamin B-12. Credit for its isolation is shared by Rickes et al., and Lester Smith, who worked simultaneously and independently. The name "vitamin B-12" was next in the list to the now-discredited vitamins B-10 and B-11, which were probably folic acid. Vitamin B-12 is also commonly known by its trivial scientific name "cobalamin."

Subscripts were formerly used to designate the various members of the vitamin B complex before chemical identification: thiamine was "vitamin B-1" and riboflavin was "vitamin B-2." Many of them as noted for B-10 and B-11 were not substantiated. "Vitamin B-6" has persisted, probably because it refers generically to pyridoxine, pyridoxal, and pyridoxamine. The name "vitamin B-12" has passed into wide popular and commercial use.

The first publication by the Merck group (Rickes et al. 1948) did not state the source of vitamin B-12, and probably many readers assumed it was from liver. Later, Merck revealed that it was from *Streptomyces griseus*.

Ellis, Petrow and Snook, of British Drug Houses Laboratories in England, also reported (Ellis et al. 1949) the isolation of crystalline anti-pernicious anemia factor from liver. William Shive (1950) discovered a test for anti-pernicious anemia factor using *E. coli* in his inhibition analysis procedure, in which growth of *E. coli* was inhibited by sulfanilamide. He

found that either methionine or anti-pernicious anemia factor would modify this inhibition on a restricted culture medium. The test responded to 0.05 g/L of vitamin B-12 (erythroin). He found that anti-pernicious anemia factor had a red color and he therefore named it *erythroin* (Shive 1951), but this name did not pass into use.

Shive et al. (1948a) isolated thymidine from liver, using their inhibition analysis procedure. They then showed (Shive et al. 1948b) that thymidine would replace vitamin B-12 in the nutrition of LLD, and they concluded that vitamin B-12 functions in the biosynthesis of thymidine.

Once the red color of anti-pernicious anemia factor had been recognized, microbiological assays were no longer needed. Indeed, as mentioned by Ahrens, Rickes and Folkers used the red color and Lester Smith isolated it on the basis of its red color alone. Smith noted that two forms of the red substance were obtained by chromatography (Smith 1948). We confirmed this in our laboratory and found that two red bands were produced on a silicic acid column, as used by Smith, when 15-unit liver extract was chromatographed in a single pass on the column. By using this procedure with *Streptomyces aureofaciens* as a source, we isolated two crystalline forms from these two bands (Pierce et al. 1949). One was cyanocobalamin (vitamin B-12) and the other was hydroxocobalamin (vitamin B-12a or B-12b) which is produced when cyanocobalamin is exposed to light (Emerson and Folkers 1951), thus solving the "matter of the two red bands."

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