OBITUARY

In Memoriam: James R. Brown (1930–2011)

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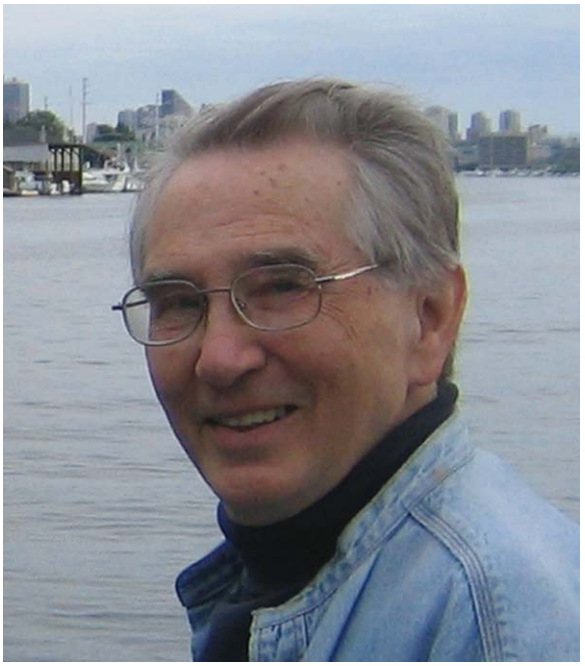

Jim Brown, a pioneer in the art of sequencing proteins in the pre-DNA era, has died at the age of 80. Jim received his Ph.D. at the University of Washington under the tutelage of Hans Neurath at a time when Seattle was the hub of the protease world for determining the structural and mechanistic basis of catalysis, structure, and mechanism. From there, Brown went to Cambridge (England) for postdoctoral work with Brian Hartley, where he worked on porcine elastase, the sequence of which provided more raw materials for an insightful *Nature* paper in 1965 on the evolution of serine proteases.

However, it was an ingenious method for assigning disulfide bonds that heralded Jim's first major triumph. The medium of the day in protein sequencing was Whatman 3M filter paper, squares or strips of which were used for paper chromatography or electrophoresis or combinations of the two. Vernon Ingram had introduced two-dimensional "fingerprints" for separating peptide fragments after a protein was digested by various means, electrophoresis being conducted in one dimension and chromatography in other. Other workers introduced "diagonal methods" in which the same conditions were employed in both of two dimensions after turning the paper 90°, some chemical or enzymatic modification being conducted in the interval that would lead to a predictable change in mobility for some of the peptides.

Jim Brown took the procedure to a new level of sophistication, however, one that allowed the determination of disulfide assignments in large and complex proteins. He did this by subjecting digests separated on paper electrophoresis directly to performic acid vapors, thereby oxidizing every disulfide to a pair of negatively charged cysteic acids. Typically, a pepsin digest was electrophoresed in one direction, after which a paper strip was cut off and exposed to performic acid vapors for about an hour. After a suitable period of aeration, an old fashioned sewing machine was used to stitch the paper strip to a new square of paper and electrophoresis conducted in the second dimension. Unchanged peptides ran along the diagonal, but all those with cleaved disulfides gave rise to a new pair of peptides running off the diagonal.

The break-through nature of the method was attested to the fact that Brown first reported it in a Biochemical Society meeting in July 1963, and the disulfide pairings of half a dozen proteins—

including immunoglobulins, lysozyme, and several proteases from the Hartley lab and others—were reported before the full publication in all its excruciating detail appeared in the *Biochemical* Journal in 1966.

After a second postdoctoral stint, this one in Israel, Brown moved to the Chemistry Department at the University of Texas, Austin. There, in a virtually single-handed effort, he determined the amino acid sequences of bovine and human serum albumins. At the time, 1974 and 1975, these were among the longest sequences determined at 590 (bovine) and 585 (human) residues, respectively. Albumin was also extremely disulfide-rich, and not surprisingly Brown exploited his early successes by using diagonal methods.

But the big surprise was what those of the sequences revealed. Assembling the peptide overlaps in those precomputer days was a formidable challenge. Jim had typed up the sequences of several dozen peptides and cut them into individual strips. He was lining them up on his living room floor when, in a revelatory moment, he realized that the structure was triplicated! This was a totally unexpected finding.

During this period, Brown also supervised the work of several graduate students, the theses of which dealt with the complete sequences of horse, pig, and chicken albumins. Together with the bovine and human sequences, these data provided early insight into the evolutionary history of this protein.

The advent of DNA sequencing in 1978 greatly changed the approach to finding protein sequences, and persons like Jim Brown, a protein sequencer enthusiast who was happiest in the lab watching blue ninhydrin spots appear on a chromatogram, began to fade into the background. But survivors of the era remember him and his strategies with great fondness.