

# LIFE HISTORIES OF SENIOR IHSS MEMBERS

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## MEMORIES OF CHARACTERIZING HUMIC NITROGEN 50 YEARS AGO

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I graduated from the University of Glasgow in Scotland in 1944 with an honors degree in organic chemistry and spent another year there as a Carnegie Research Scholar synthesizing compounds for cancer research. I was then persuaded by the Director of Rothamsted Experimental Station in Harpenden, England, to join the Chemistry Department at Rothamsted to initiate a program of research to characterize the organic substances in soil. I should have had my head examined !

Rothamsted had a wonderful science library and I spent many hours there reading everything I could find concerning the organic material in soil. I was very disturbed to discover that less than 1% of the organic material in soil had been identified chemically despite more than 100 years of research and to realize that none of the techniques available to organic chemists at that time were likely to be of significant value if applied to the humic acids and other complex organic materials in soils. And it was very discouraging to find numerous USDA Bulletins reporting failure to isolate more than very small amounts of identifiable organic compounds from very large amounts of soil. By good fortune, the techniques of paper and gas chromatography were developed in England shortly after I started to work at Rothamsted, and I decided to try to adapt these techniques for research on soil organic matter.

No equipment for paper chromatography was available when I started to investigate the potential value of this technique for research on soil organic matter and most of my early work was conducted using primitive equipment, including large drain pipes and long sections of glass tubing modified to serve as troughs for organic solvents and hold the edges of large sheets of Whatman filter paper. It was not possible to handle these large sheets without assistance, and I had no laboratory assistant at that time. I had, however, a very talented girl friend, Mary Williams, who worked in the Microbiology Department at Rothamsted, and she provided valuable assistance when help was needed in my chromatographic work. During this work we were exposed many times to air saturated with collidine, lutidine, and other organic solvents later classified as dangerous chemicals, but we survived, got married in 1950, and are still around more than 50 years later. We thought we had discovered a new method of fingerprinting when we sprayed two-dimensional paper chromatograms with ninhydrin to locate amino acids and found that our fingerprints on the paper showed up very clearly on the sprayed chromatograms, but a couple of researchers in London were faster in reporting this observation. Recent TV news programs indicate that ninhydrin is still being used for fingerprinting in criminal cases.

My plan was to hydrolyze the protein material in soils with 6N HCl and identify the amino acids thus released by paper chromatography. The major problem was that the inorganic material in soil hydrolysates prevented application of paper chromatography, but this problem was overcome by a technique that permitted removal of salts without loss of amino acids and I was able to obtain excellent chromatograms from all soil hydrolysates studied. Rothamsted was famous at that time for its many visitors, and one day I had a visitor - I did not catch his name - who had heard that I was using paper chromatography. Being full of enthusiasm about this technique, I gave him a pep talk on its potential value. I saw him later that day at afternoon tea on the lawn of Red Gables at Rothamsted and was mortified to learn from a colleague that my visitor was one of the inventors of paper chromatography (he subsequently received a Nobel Prize).

During 1948 and 1949 I studied the amino acid composition of 10 different soils by paper chromatographic analysis of acid hydrolysates of these soils. The following 20 amino acids were detected in every hydrolysate examined and estimated semi-quantitatively: phenylalanine, leucine, isoleucine, valine, alanine, glycine, threonine, serine, aspartic acid, glutamic acid, lysine, arginine, histidine, proline, hydroxyproline,  $\alpha,\epsilon$ -diaminopimelic acid,  $\alpha$ -amino-n-butyric acid,  $\beta$ -alanine,  $\gamma$ -aminobutyric acid and tyrosine. Glucosamine and methionine sulfoxide were detected in most of the hydrolysates. No free amino acids could be detected in any of the soils studied.

The detection of glucosamine suggested that a significant amount of the nitrogen in soils and humic acids might be in the form of amino sugars. To investigate this possibility, I initiated work with Ken Shaw to estimate amino sugar-N in acid hydrolysates of soils by two techniques, and this work showed that 5-10% of the nitrogen in the soils examined was in the form of combined amino sugars (J. Agr. Sci. 1954, Vol. 44, pp. 152-159). I published my work on the amino acids in soils in a 1950 issue of the Biochemical Journal (Vol. 50, pp. 123-145). This publication created a great deal of interest in Europe and I received invitations to visit and work at several research centers. I accepted an invitation by Professor Wolfgang Flaig to initiate research on humic acids using paper chromatography at the Institute for Soil Biochemistry in Volkenrode, a research center near Braunschweig in West Germany, and my wife and I spent four happy months there in 1954. I then made a tour of German research centers and was astonished by the tremendous interest in humic acids there because there was very little interest in Britain at that time. Two publications concerning

humic acids emerged from my work in Germany. I started work to identify the nitrogen in humic acids in 1949 and published preliminary results of this work in 1952. A full report of the work was published in the Journal of Agricultural Science in 1955 (Vol. 46, pp. 247-256). In this publication I reported research to characterize the nitrogen in humic acids isolated from 11 soils by the customary sodium hydroxide extraction procedure and by the neutral pyrophosphate procedure I introduced in 1949 (J. Agr. Sci. 39:274-279). I determined the amounts of total N,  $\alpha$ -amino acid N, amino sugar N, and ammonia N in 6N HCl hydrolysates of these humic acids and identified the amino acids in the hydrolysates by paper chromatography. This work showed that humic acids isolated from alkali and pyrophosphate extracts of the same soil differed markedly in total nitrogen content and in nitrogen distribution after acid hydrolysis. The alkali-extracted preparations had a higher N content and a higher proportion of acid-soluble N and  $\alpha$ -amino acid N. At least 31-48% of the N in the alkali-extracted preparations and 20-35% of the N in the pyrophosphate-extracted preparations was in the form of amino acids, and 3-10% of the N in the preparations was in the form of amino sugars. Paper chromatographic analysis of acid hydrolysates of the preparations indicated that the protein materials in humic acids extracted from different soils by alkali or pyrophosphate were similar in their amino acid composition. The following nineteen amino acids were detected in every hydrolysate examined: phenylalanine, leucine, isoleucine, valine, alanine, glycine, threonine, serine, aspartic acid, glutamic acid, lysine, arginine, histidine, proline, hydroxyproline,  $\alpha$ -aminobutyric acid,  $\beta$ -alanine,  $\gamma$ -aminobutyric acid and tyrosine. Amino sugars and oxidation products of cystine and methionine were also detected in every hydrolysate examined and a substance provisionally identified as  $\alpha,\epsilon$ -diaminopimelic acid was found in some of the hydrolysates.

I concluded in 1955 that the nonhydrolysable nitrogen in humic acids must be largely, if not entirely, in the form of heterocyclic nitrogen compounds. I did not attempt to identify this nitrogen because no techniques were available for such an investigation and I saw little prospect of techniques being developed in the near future. I speculated on possible structures of these compounds and how they may be formed in soils in a chapter on organic nitrogen in soils I wrote for the monograph entitled "Soil Nitrogen" published by the American Society of Agronomy in 1965.

During my early years at Rothamsted I devoted a considerable amount of my time to development of reliable analytical techniques for research on soil organic matter because no such techniques were available at that time. In searching the literature for potentially useful techniques I came across the publications of Donald D. Van Slyke, who developed numerous methods for blood analysis while working at the Rockefeller Institute in New York. I greatly admired his work because he subjected the methods he developed to very rigorous tests for both accuracy and specificity and was an exceptionally thorough researcher. He developed a highly specific and accurate ninhydrin method for determination of  $\alpha$ -amino acid nitrogen in blood, and I adopted this method for determination of  $\alpha$ -amino acid nitrogen in hydrolysates of soils and humic acids. He had a big influence on my career because his work inspired me to try to develop numerous methods clearly needed for research on soil organic matter. He worked at the Rockefeller Institute in New York and I regret that I did not have an opportunity to meet him when I arrived in New York in 1957 with a Rockefeller Fellowship allowing me to spend one year visiting research centers in the USA. During that year I was offered a tenured position at Iowa State University and I have not worked on humic acids since that time.

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