INTERVIEWS: JMW

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Meeting with Biochemists to determine the best method for amino acid analysis of grains.

JMW, Dr. Norman E. Borlaug, and Dr. Edwin J. Wellhausen met with Dr. Aaron M. Altschul, Dr. Robert H. Barker, Dr. Kenneth A. Gilles, Dr. Edwin T. Mertz, Dr. Stanford Moore, Dr. Yeshajahu Pomeranz, Dr. George F. Sprague, and Dr. C. Glen King for a discussion on the best methods for analyzing various grain hydrolysates for amino acid content.

Borlaug and Wellhausen outlined the problem facing the group. There are 12,000 lines of corn at the International Center for Corn and Wheat Improvement in Mexico City. These roughly consist of 250 races. It would be necessary to analyze about 2500 of these lines after selection had been made based on other known characteristics. At the same time, in wheat, it would be necessary to analyze 1,000 lines just to determine protein and amino acid content. During a period of crossing, Borlaug would require 500 analyses. They stated that in all probability a similar situation would exist with rice, sorghum, and the millets. The problem breaks down into two phases: one, to screen all lines of the various grains for total protein and the specific amino acids; second, and more difficult, to analyze the grains rapidly to permit the plant breeders to make proper crosses at least twice a year. This would mean doing several hundred samples within a period of perhaps two weeks. All agreed at the meeting that the investigations should concentrate on those lines which have high protein and which must have high lysine. It followed, therefore, that the most rapid method for determining protein and total nitrogen should be utilized. Various views were expressed, but it was agreed that in all probability the simplest method would be that of using the automatic Kieldahl analytical equipment produced by Technicon Company. This would permit the investigator to run about 40 samples an hour for total protein. The problem with the amino acids is somewhat more complicated. Everyone agreed that it would not be fruitful to follow up on lines without a high lysine content, and that the most sensible approach, after determining total protein content, would be to choose lines with a high lysine for crosses and use only these in investigations for total amino acids.

Dr. Stanford Moore, of The Rockefeller University, and certainly the most eminent authority on amino acid analysis, stated that there were three possibilities for obtaining the necessary data. First, he talked of doing only lysines in a large series of samples. By using chromatography, in this case either a Beckman or a Technicon analyzer, one could do 20 samples for lysine per day on a special apparatus, in other words a short column. This could be speeded up by using three short columns, thus getting three per hour or 72 per day, working on a 24-hour basis. Gas chromatography is a possibility

and a new apparatus for this purpose will be described later in the diary note. A second approach would be a microbiological assay, and thirdly, a cholerometric method, which as yet is not available. However, Professor Barker is now doing research to develop a cholerametric method and it was his opinion that they should have a method that would do at least lysine fairly rapidly. If such a cholerametric method in non-aqueous solutions is developed, then one should be able to do hundreds of samples per day. Dr. Pomeranz of Kansas State University had had the greatest amount of experience in microbiological assays. He described them and stated that it would be possible to do a substantial number of microbiological assays per day if one were looking only for lysine. Moore had the feeling that the type of data that would be obtained from microbiological assays would not be precise enough to be of any value to the plant breeder and pointed out, as did Altschul, that too often in these cultures a mutant occurs so that the result becomes meaningless. Pomeranz agreed that this was the case and suggested microbiological assays only as a possible, rather rough, screening method to determine gross data on the total amino acid picture. In the end it was decided that the only reasonable method would be to use amino acid analyzers of one variety or another.

If one desired total analysis for all the amino acids in grain, then a different level of production must be anticipated. Moore stated that if one were looking for several amino acids to include lysine, tryptophan, and the sulfur amino acids, the following rate of output might be anticipated:

a. A 60 to 70 minute run on the analyzer for sulfurs, following acid oxidation and hydrolysis. If one were to use three columns, this would in effect turn out about 72 per day.

b. The Kineurenin system for tryptophan, using alkaline hydrolysis, will give you a 90 per cent recovery rate, and is possibly the best at the moment. This, Moore anticipated, would again require 60 to 70 minute runs. It was his opinion that, in an eight-hour day, one could anticipate doing five samples for sulfur amino acids, five samples for tryptophan, and about 20 samples for lysine. Of course, the daily output would be tripled in a 24-hour day. Turning to other important amino acids, one could anticipate running five samples per eight-hour day for threonine or a total of 15 for a 24-hour day. The same total would be reached for isoleucine and leucine, while obtaining only the ratio of one to the other.

The various models of amino acid analyzers now on the market were discussed by the biochemists who were using one or the other of them. There was general agreement that the Beckman Spinco unit is the best currently available if one wants sharp curves and highly reliable data at the level

of 1% per cent accuracy. The Technicon was felt to be somewhat less satisfactory because it gives less resolution. However, Altschul from Tulane is using a Technicon and says that he gets quite good results in the work with oils. The Phoenix unit is said to be good but not as good as the other two. The fourth unit is one which will be described in greater detail later and is just currently being developed by the United Aircraft Company in Sunnyvale, California. The final agreement was that, if one had unlimited funds and had to buy a unit at this precise moment, the Beckman Spinco would be chosen, costing about $36,000 including spare parts and integrator. However, the Technicon would cost about half this much and there was agreement that in all probability this unit would do the job needed and would be the one of choice except for the possibility of using the gas chromatography setup of United Aircraft. As far as integrators were concerned, in order to obtain print-out of the results, Moore uses the infratronic unit which is produced in Houston at a cost of $8500. This integrator will work with both the Spinco and the Technicon, as well as the Phoenix analyzers. Spinco has recently produced an integrator at a cost of $6200. Moore is currently testing it in his laboratory and does not feel that it is as reliable, and it is more difficult to maintain, as the infratronic. Technicon utilizes a planimeter which costs $3000. Moore does not feel that this is a good instrument for this work, nor is it reliable.

Moore and the others emphasized the necessity for having ample spare parts on hand and $2000 per year for supplies and spares should be added to the basic cost of the amino acid analyzer in estimating budgets. It was also agreed that since the units will be utilized in areas where electronic services are not always readily available it would be wise to have at least two units, both to speed up the operation and to know that one would always have one operating in the case of some sort of breakdown. In the case of Mexico City, it was agreed that at least three would be needed. Questions were raised as to how one could train the proper number of technicians for an investigation of this size. Dr. Mertz stated that he has a very able graduate student who has run his analyzer for several years and could be made available to help in the training program. The present plan, and a highly desirable one, is that this young man will go to Mexico City for a period of two years where he will run the analyzers for Wellhausen and Borlaug and at the same time train technicians, who will be sent from India, Los Banos, and other areas where the investigations will be going on. It was also emphatically stated that the technician who is going to run the chosen unit should disassemble it in the United States, reassemble it, and run it for a period of a week to ten days so that he is quite certain it is operating properly before sending it abroad. For the most part this will be academic, since each of the companies requires that the individual responsible for the unit spend a period of two weeks in their factory taking courses in operation and maintainence of his own unit, as well as running it, before it is shipped overseas.

Subsequent to the meeting, both Drs. Moore and Mertz followed up on the various units that had been considered. Mertz made the very sensible suggestion that his graduate student take grain hydrolysates and visit laboratories where the most modern unit of Technicon is in operation and run the hydrolysates through the Technicon to determine how accurate and reliable it is in comparison to the Beckman Spinco which Mertz is currently using. The Technicon people were contacted and we discovered that there were four of the most modern units in other departments at Purdue, one of them being in the laboratory of Henry Kaufler, who is doing pig feeding to test the new varieties of opaque-2 maize. However, all of these units were fitted with only the long columns which would only produce one sample per day and would obviously not be satisfactory. The Technicon people air shipped two short columns to Mertz who put them on the unit in Kaufler's laboratory and determined whether they were satisfactory for running the maize samples at least. Later the young man will go to United Aircraft and run samples through their unit.

Moore followed up in two respects. He found people using the automatic Kieldahl unit of Technicon and wrote to say that this is very satisfactory and is capable of putting out the number of samples claimed by Technicon. In the second instance, he got in touch with the people of United Aircraft's Division in Sunnyvale where the new gas chromatography unit had been developed to analyze amino acids in blood sera taken from astronauts in the space program. They are doing studies there on the use of straight amino acid diets in space. This unit is a compact one, relatively simple, fully automated, using a gas chromatography unit, and has a print-out integrator as an integral part of the unit. The samples are placed in a very small plastic container and inserted into the machine, following which all the analysis is fully automatic. The amino acids are inverted to volatile derivitives. The advantage to the approach is a very high sensitivity and the fact that you can use very small amounts of the sample. Dr. Winitz, who is doing the work at the United Technology Center, has been able to determine three amino acids in one-tenth of a drop of blood. The unit will not, at the present time, determine the level of arginine. However, this is not important in the analysis of foods. Cysteic acid from cysteine is not yet aminable to analysis, and this is important in nutrition. Winitz expects to produce a publication about the machine within the next three months. The equipment, so far, has not been tested with the hydrolysates of cereal grains, but this can be remedied by sending Mertz's young man there to run a few samples through the unit. One really appealing aspect of this unit is the fact that it is so simple that it can be operated by a secretary in United Aircraft after a few days of instruction and practice. This would be a great advantage in use in areas where the technicians are not altogether adequate. JMW called the engineer in charge of the project at United Aircraft. He said that they had been attempting to get Perkin-Elmer to produce and market the instrument, but Perkin-Elmer had been making some unusually high demands for their services. Therefore, the United Aircraft Corporation decided that, if Perkin-Elmer did not come to terms rapidly, they would produce the instrument themselves, which should market at something around $8,000, a distinct advantage over any of the other amino acid analyzers. They hope to be in production within the next month to six weeks, and United Aircraft felt that they could begin to deliver units some time around October of 1966. This would seem,

therefore, to be the unit of choice, and after consulting with Mertz, Moore, Altschul and others, it would seem to JMW that the RF investigations should rely upon the Technicon automatic Kieldahl analyzer, upon the Technicon auto analyzer for broad spectrum chemical work, and upon the United Aircraft gas chromatography unit, which will be available within a reasonable period of time. One would also want to have the infratronic's integrator with automatic put-out, since this reduces appreciably the time spent by the technician and investigator.

Finally, there is a very rapid method of determining the level of lysine, if this is the amino acid of interest, and would be very useful indeed in at least sorting out and determining which lines gave promise of high lysine and higher protein content. The method was developed for Merck and Company to use as a quality control instrument in their commercial production of lysine. It does not measure the lysine directly but relies upon measuring decarboxylase which indicates the level of lysine. It should be possible to analyze about 40 to 50 samples per hour with this instrument. Mertz followed up by getting the technical details as well as the information contained in the original description by Scheuerbrandt in an article entitled, "Automatic determination of the decarboxylase for aromatic amino acids in mouse liver," published in the Analytical Biochemistry, volume 13, No. 3, December, 1965. An additional article is that by Schaiberger and Ferrari entitled, "Automatic, enzymatic Analysis for L-Lysine via decarboxylation." This was published in the Annals of the New York Academy of Science's Volume 87, Article Two, pages 890-893, July 22, 1960. Mertz has already set up a makeshift apparatus in his laboratory and has found that the decarboxylation reaction is precise, rapid, and certainly is one that can be used on grains with great ease, and with little technical skill. Therefore, this would be the instrument of choice for the rapid screening of the lines, whether they be rice, wheat, maize, sorghums, or millets.