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DR. CARPENTER: I would like for you to remain for a brief discussion. We have no formal program for lunch and so I would like to call on Dr. A. M. Pearson to entertain any questions for 10 or 15 minutes. Dr. Pearson.

DR. A. M. PEARSON: Thank you, Mr. Chairman. In going back to some of our experiences in gas chromatography, I think they began in about 1956. So we have been doing work in this area for
quite some time although our emphasis has been somewhat different from the speakers today because we have been primarily interested in flavor compounds. Flavor chemistry is much more difficult perhaps than where you know the compound that you are working with. In flavor chemistry we know little about the compounds we are working with and it is much more difficult to isolate and characterize compounds with which you are not acquainted. We had four excellent papers here dealing with gas chromatography. I think the paper given by John Sink would indicate that it is a useful instrument. But I think we have a problem here which we do need to discuss. Oftentimes, the most difficult thing is isolation of the sample which you wish to analyze. I think this is an area that could stand some discussion today. The paper by Dr. Stalling certainly made an elegant one for discussion. In fact, we have had two papers today which showed methods of determining compounds which at one time were thought impossible to be determined by gas chromatography; namely, the amino acids and the triglycerides. We have made the statement at one time that they could not be analyzed by gas chromatography. Now the methods have been developed whereby you can use gas chromatography and they have been very elegantly presented to us today by Dr. Stalling and by Dr. Kukis. Dr. Lisk, of course, gave us a very interesting talk dealing with gas chromatography of the pesticides and I was particularly interested in the instrument which he described. I think it would be of some interest if he would give us the name of this instrument and where it can be obtained because it seems to be most useful. I think we could also ask him in this regard, does it work in combined sulphur-containing compounds; that is, could it be used to determine the levels of sulphur compounds just merely as an elemental sulphur analysis? I would like to turn some time over to you for questions although first I would like to ask Dr. Lisk if he would speak to us very briefly concerning the questions which I have asked.

DR. LISK: As for the source of this device, the original paper by Cook was published in the Analytical Chemistry in 1965. I don't have the page number, but can get it for anyone who wants it. I am right next door about 100 feet away from this building so I would be glad to show you the device and we can give you facts and figures about it. Now your second question had to do with determining elemental sulphur in combined compounds. If the compound contains sulphur and if it will go through a gas chromatography column you will see this sulphur band emission and you can do compounds this way. In fact, I had a slide (I did not show it today because of lack of time) in which we followed the sulphur line instead of the phosphorus. The reason we chose the phosphorus line is because the phosphorus gives us the sensitivity that corresponds to about one times ten to the minus 13 grams per second. In other words, if you inject a tenth of an anagram of phosphorus you can see it. The sulphur line is about ten to a hundred times less sensitive. So therefore, the ability to look at either sulphur or phosphorus in one end of a molecule will depend on the concentration. You could surely do it on a formulation analysis. But for residue analysis, you might have to make two separate injections,
one in which the phosphorus peak would go off scale, but you could see the sulphur peak. So you make your choice for analysis just like we are based on the strongest emission you get. This is one of the unfortunate parts of emission work. It is an old technique and many of you are probably familiar with it or heard of it, but the emission that you get off will vary in strength depending upon the probability of transition taking place. In other words, when you excite an atom, if a certain electron is being kicked up into a higher orbit and dropping back and it is a very probable occurrence, then you will have a large number of these atoms undergoing this transition. Therefore, you will get a strong signal and therefore have a sensitive response for it. If it is less probable, then your sensitivity will vary as your probability varies. With phosphorus, as I say, we can do parts per billion of a compound of organo-phosphorus and insecticide in a crop. This is plenty sensitive for residue analysis. For sulphur you probably would have to be satisfied with 10 parts per billion. Iodine is about ten times less sensitive than phosphorus. You could probably do ten parts per billion of an iodinated compound. But to answer your question, if the compound contains sulphur and if it will go through the chromatographic column you can sit on the sulphur line and do it. By using a series of standards you can tell how much is present.

DR. PEARSON: What is the name of the instrument or doesn't it have one yet?

DR. LISK: Well, I guess it depends on how long you want to talk. Cook has named it micro wave emission detector and some people just call it the micro wave detector. I think the micro wave detector emission is the most descriptive because this is actually what you are doing. You are using micro wave energy to cause excitation and then looking at that emitted energy.

DR. PEARSON: Is it commercially available?

DR. LISK: There are some companies that have visited Cook's lab and have also seen our lab but have not committed themselves as yet as to whether they are working on it or are going to come out with it or not. We suspect that it will come out shortly though.

DR. PEARSON: Thank you. Are there other questions? I would like to ask Dr. Kuksis very briefly to comment on the order in which the compounds come off. I noticed, if you give these peak numbers, do the same numbers always correspond to the same compound?

DR. KUKSIS: Yes and no. I should say today that it's very simple. We just count the carbons in the fatty acids and add all of them up. When correlated to a number of other compounds one could certainly correlate to any kind of glycerides one has and to a number of other compounds. But when one goes to stearyl esters and what are you going to name there? Are you
going to count the number of stearyls, the carbons in the stearyls or are you going to count the carbons in the fatty acids? Because they are eluted ahead of triglycerides and in some cases overlap the triglycerides over approximately the same carbon number. For instance, cholesterol arachadonate 47 (27 carbons in cholesterol, 20 in arachadonic acid). That overlaps the C46 that's with the triglyceride that will contain any combination of fatty acids that will give a total number of 46 carbons. So we counted all of the carbons there. We did the same thing when we came to stearyl. Again, one can say for instance, cholesterol 27 carbons combestol 28, berosterol 29, they run all ahead of tridecanoin which is C30. But when one gets down to fatty acids that we can run in the front of the chromatogram, we will have to count whatever we had in the event of monoglycerides. We ended up using the same numbers for three fatty acids as for monoglycerides. Diglycerides we counted as stragglers as three diglycerides. But the problems really arise when you use monoglyceride acetates or when you use diglyceride acetates. Does this satisfy this?

DR. PEARSON: Yes, thank you very much. I think this answers the question. Are there other questions? I wonder if Dr. Elston would comment on the method that they have used for the preparation of methyl esters.

DR. ELSTON, Harvard University: Currently I wish I could give you a good method of preparation of methyl esters. We have extended the range of our detection to the point where we are getting a lot of trouble. The best I can describe is oxidation products. We are using the old procedure developed by Dr. Ahrens at the Rockefeller Institute. So this is simply refluxing the lipid mixture with methanol-sulfuric acid or hydrochloric acid, neutralizing with sodium bicarbonate and extracting in pet ether. I think Dr. Dugan from Michigan State recently developed a procedure that seems to be good for a large quantity. Is this what you were referring to, Dr. Pearson?

DR. PEARSON: Yes.

DR. ELSTON: There is a low temperature of methylation in that procedure (I am not exactly sure of its temperature) but it's usually on a dry ice ethanol bath. It involves dissolving the glyceride or the lipid mixture in either diethyl ether, heptane, hexane or some such solvent, adding concentrates to sulfuric acid at about $2\frac{1}{2}$ mls of concentrated sulfuric acid to 20 mls of this pet ether. I should not say pet ether you have some sulfur there; diethyl ether or heptane. He stirs this under nitrogen for 10 or 15 minutes and then neutralizes with sodium hydroxide. At this point he allows it to warm at room temperature so that the salt form will stay in solution. It is easily separated in a separatory funnel and I believe he washes the mixture three times. I am not sure how good a micro procedure this is. It is a very rapid macro procedure and I have used it with quite good success. It takes very little equipment.
DR. PEARSON: Thank you, Charlie. Any other questions.

A comment from Dr. Kuksis.

DR. KUKSIS: I would like to amplify my comments here about fatty acid chromatography. In respect to the preparation of fatty acid methyl esters, I would like to call your attention to the decision of the American Oil Chemists' Society because they have not proposed that two percent sulfuric acid-methyl alcohol should be used for the methylation of free fatty acids or for the transmethylation of fatty acid esters. This one is described in the January, February or March issue of 1966 of the Journal of the American Oil Chemists' Society. They also give some procedures. It's sort of a combined effort of a committee and five or six cooperating laboratories to decide how this should be done. I would like to say that in our experience the sulfuric acid methyl system has proved to be far superior to the HCL methyl system. You can over methylate with sulfuric acid quite readily without any noticeable destruction of fatty acids. When one works with very small quantities of fatty acids or fatty acid methyl esters, it is of great interest with work in thin-layer chromatography, to take these bands off the thin layer plates, one can't possibly be swabbing the plate with additional solvent and then complete the methylation or transmethylation in the presence of the silica gel. This sort of transformation will take place very effectively in the same methylating agent. Say we have been using 10 percent by weight of sulfuric acid methanol, one has to increase the reaction time to complete the methylation maybe two hours in the absence of silica gel. In the presence of silica gel, you may have to run overnight and if you have some possible lipids present like sphingomyelin you would not get a complete yield of the fatty acid ester unless you increased the temperature from 85 degrees to maybe 110 degrees as Feld and Reiser reported last fall.

DR. PEARSON: I would like to ask Mr. Stalling a question concerning the relative time in making analysis of the amino acids for the esters of the amino acids as compared to that when you use your ion-exchange column.

DR. STALLING: In reference to the time required for analysis of the amino acids in the procedure we are presently employing, there is a half hour involved in esterification and then two and a half hours for interesterification which gives a total amount of about three hours. The acylation can be done in about 5 or 15 minutes depending upon the conditions you are working with. The operator or technician who is doing the analysis can be doing six or twelve amino acid samples. If he starts in the morning he can, on one chromatograph, run eight protein hydrolysates in an eight hour working day and we feel this is representative. If you are interested in the lysine content of a feed or if you are interested in methionine or certain of the amino acids, you can get these analyses within 5 minutes estimation time on the chromatograph, which means you can run twenty samples an hour.
DR. PEARSON: Thank you very much. Now I will turn the program back to our chairman. I think we have used all the time he has allotted us and I am sure we all want to eat our lunch.

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DR. CARPENTER: Thank you, Dr. Pearson. For our lunch we will be at the Dairy Bar in Stocking Hall. O.K. we are adjourned. Thank you.

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