RESEARCH INVESTIGATION OF

ELECTRICAL DISCHARGE FRAGMENTATION FOR

PROTEIN STRUCTURE IDENTIFICATION

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ELECTRICAL DISCHARGE FRAGMENTATION OF BACTERIA

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1. Introduction

The employment of fragmentation methods for the identification of substances was initiated by the use of mass spectroscopy and has increased many fold during the past several years. Much of the recent increase is due to the development of atmospheric pressure fragmentation instrumentation based on gas chromatography or infrared analysis. Originally, the use of this atmospheric pressure fragmentation process was concerned primarily with non-life associated materials such as polymers. The extension, however, of this system to life-related materials was spurred by the increased biochemical research and the space programs. Because of its speed, sensitivity, and capability of separating complex mixtures, such as those obtained from fragmentation, gas chromatography has been employed in virtually all of these investigations of life-related materials. Since one hour or less identification time is required in fragmentation methods, whereas days are often required in biological methods of identifying life-related materials, it is easy to understand the interest in developing the fragmentation system. One of the most tedious and perhaps the most difficult identifications involving life-related materials is the labeling of unknown bacterial substances found in our environment.

The extremely interesting, and yet unanswered, question of whether life exists on heavenly bodies other than our earth can only be investigated with a rapid system. Fragmentation at atmospheric pressure is now being investigated and is the object of this study for use in the identification of life in space and in the identification of bacteria. The tremendous advantage of being able to identify bacterial strains shortly after obtaining them from a patient can be of extreme importance in diagnosis and treatment.

To date, most of the investigations for detecting extraterrestrial life as well as bacterial identification have employed thermal fragmentation. There are essentially two forms of pyrolysis exhibited by the work of Garner (1) and Reiner (2) (2a). Garner, as reported in C and E News, operated at very low temperatures, almost as a distillation procedure. Reiner employed more usual thermal pyrolysis temperatures of approximately 800°C. In the present study both of these techniques have been translated to fragmentation with electrical discharge, which has been demonstrated to be equivalent to thermal pyrolysis in fragmentation patterns but easier and faster to handle.

2. Apparatus

2.1. Chromatographic Apparatus. Because of its versatility, reliability, and its precision and sensitivity, the Beckman GC-4 gas chromatograph utilizing a hydrogen flame ionization detector was employed. The electrical discharge fragmentation accessory was employed in the analysis of the life-related materials. The upstream electrode was modified by installing a .025" diameter piece of platinum wire in the gas passage hole to serve as a point whereby the discharge could be more reproducibly located.

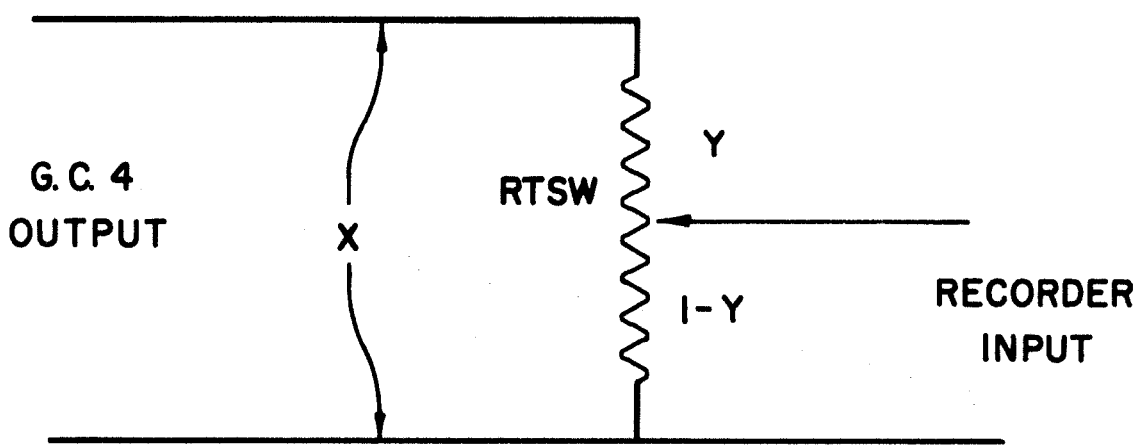
In the initial stage of the investigation an 1/8" O.D. 3% S.E. 30 on chromosorb W column of 20 feet in length was employed for separating the fragmentation products. The SE-30 column was chosen because of its tremendous temperature range capabilities. The column temperature was programmed from 0°C to 250°C, employing a liquid carbon dioxide cooling accessory unit to obtain the initial temperature of 0°C. The column was held isothermal at 0°C for 4 min. and programmed linearly at 12.5°C/min. up to 250°C, where it was held isothermally for the duration of the run.

During a second stage of this study, a Carbowax 20M capillary column .010 inches I.D. and 200 feet long was used with a 40:1 splitter; however, the capillary yielded inferior results for this application due to the fact that a large quantity

of material is dumped onto the head of the column and spreads down the column, even at a temperature of 50°C, because of the low sample capacity. This effect leads to irreproducibility of retention times as well as badly spreading the first few peaks in the pattern. Because of these factors the capillary column was removed and replaced by a 3% Carbowax 20M on chromosorb W column, 1/8" O.D. and 20 feet in length. The Carbowax column was programmed from 50°C to 200°C. The change of state of Carbowax 20 M that occurs at approximately 50°C prevented going to lower temperatures, for at this transition retention times are difficult to reproduce. Again, the liquid carbon dioxide cooling unit was employed in obtaining the starting temperature. The first few minutes of the run was isothermal at 50°C, and the program was linear at 7.5°C/min. until 200°C, and isothermal thereafter.

2.2. Recorder. The Beckman 10" recorder was employed. The ease of chart speed changing made possible the use of various speeds depending on the number of peaks occurring within a given time interval. During the initial stages of the project the recorder was employed in its normal linear response mode. It was found, however, that attenuation changes had to be made several times during the run in order to keep large peaks on scale and still enable possible quantitative measurements of smaller peaks. For this reason, the recorder was modified to provide a readout giving a compressed scale for large signals, while retaining high sensitivity and linearity for small signals; this was accomplished by installing a retransmitting slidewire in the recorder as shown in figure I. The actual function produced was $Y = X/(E_{fs} + X)$ where Y is the recorder position, X the electrometer output signal, and E_{fs} the full scale voltage of the recorder which was 1 millivolt so that $Y = X/(1 + X)$. Thus a 1 millivolt electrometer output yielded a half scale reading and a 3 millivolt output yielded a recorder position of 75% of full scale. This function made possible the observation of all peaks, large or small, with a single scale

FIGURE 1.



setting of 5×10^{-11} amps full scale on the electrometer.

3. Flow Patterns

Several flow configurations were employed, all involving the use of a gas sampling valve. At first, the sample loop (see figure 2) was switched into the column carrier flow system which was fed through a flow controller. This unfortunately had the adverse effect of producing a flow upset, for the sample loop was at atmospheric pressure and the column carrier stream was at column head pressure; thus, some of the flow from the controller was required to increase the pressure of the sample tube, and consequently the flow through the column fell appreciably until the pressure in the sample loop reached column head pressure. Since the effect of this flow upset could impair the reproducibility of retention times, a system was employed (see Figure 3) which compensated for the sample loop being at atmospheric pressure and made it possible to achieve retention times reproducible to better than 1%. If the pressure controller is set at or slightly below column head pressure, when the column is at the starting temperature, and the flow controller set to the desired flow, the pressure controller will immediately increase the pressure in the sample loop to column head pressure when the loop is injected into the column carrier line, thus keeping the flow constant. After the sample loop is in the column carrier line, sample is swept onto the column. Before the column temperature is raised, the pressure controller is switched out of the system to prevent subsequent flow from the flow controller into the pressure controller during the temperature program as column head pressure increases with column temperature. The flow system used for the capillary column does not involve this previously stated problem of the sample loop pressure, since the flow through the capillary column is produced via a pressure controller (Figure 4). The problem involved in employing a capillary column is one of sample capacity; thus one must split the effluent of the sample loop before it reaches the column. For this purpose

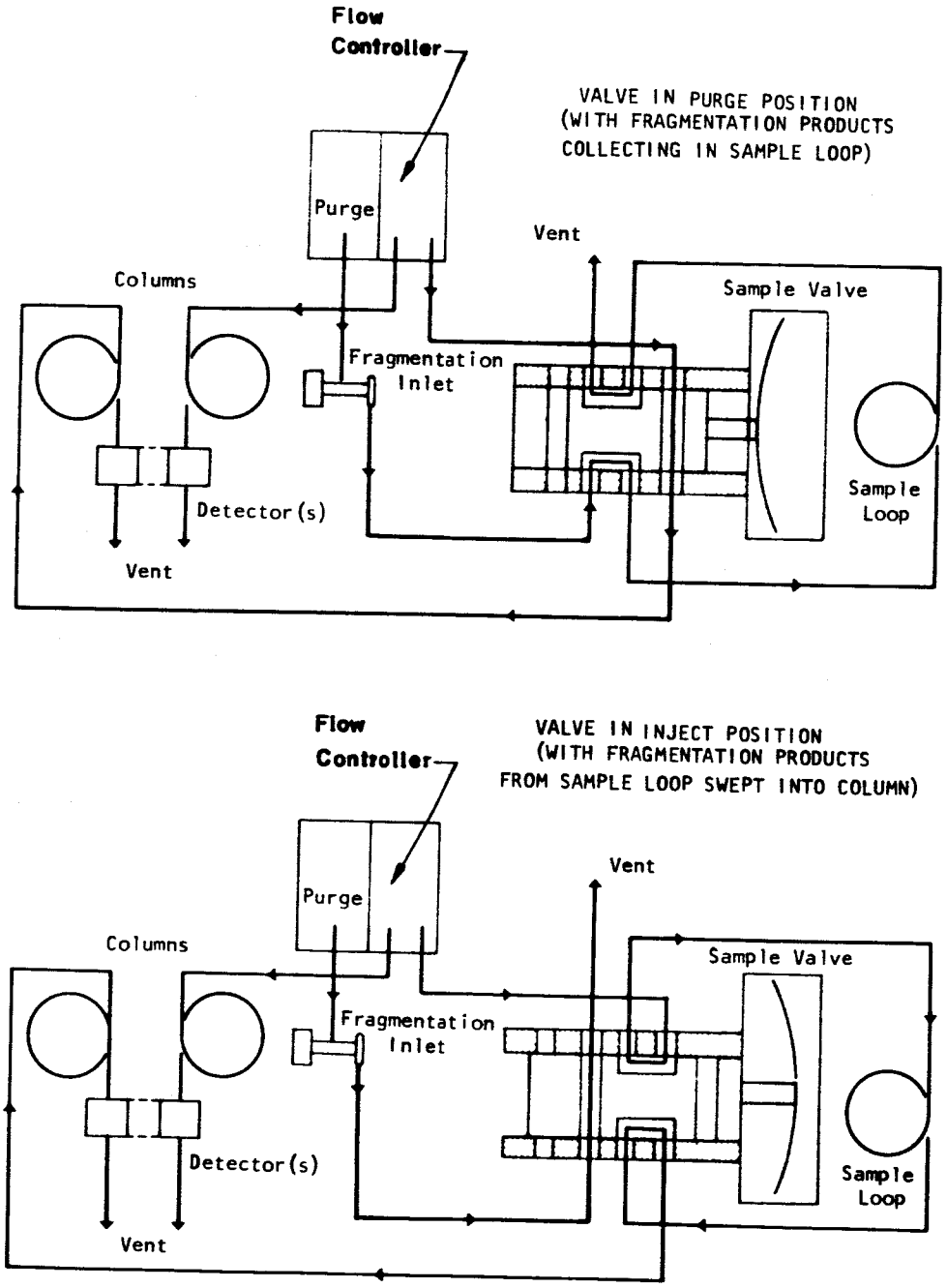

Flow Controller
VALVE IN PURGE POSITION
(WITH FRAGMENTATION PRODUCTS
COLLECTING IN SAMPLE LOOP)
Purge
Vent
Columns
Sample Valve
Fragmentation Inlet
Sample Loop
Detector(s)
Vent
Flow Controller
VALVE IN INJECT POSITION
(WITH FRAGMENTATION PRODUCTS
FROM SAMPLE LOOP SWEPT INTO COLUMN)
Purge
Vent
Columns
Sample Valve
Fragmentation Inlet
Sample Loop
Detector(s)
Vent

FIGURE 2.

FIGURE 3.

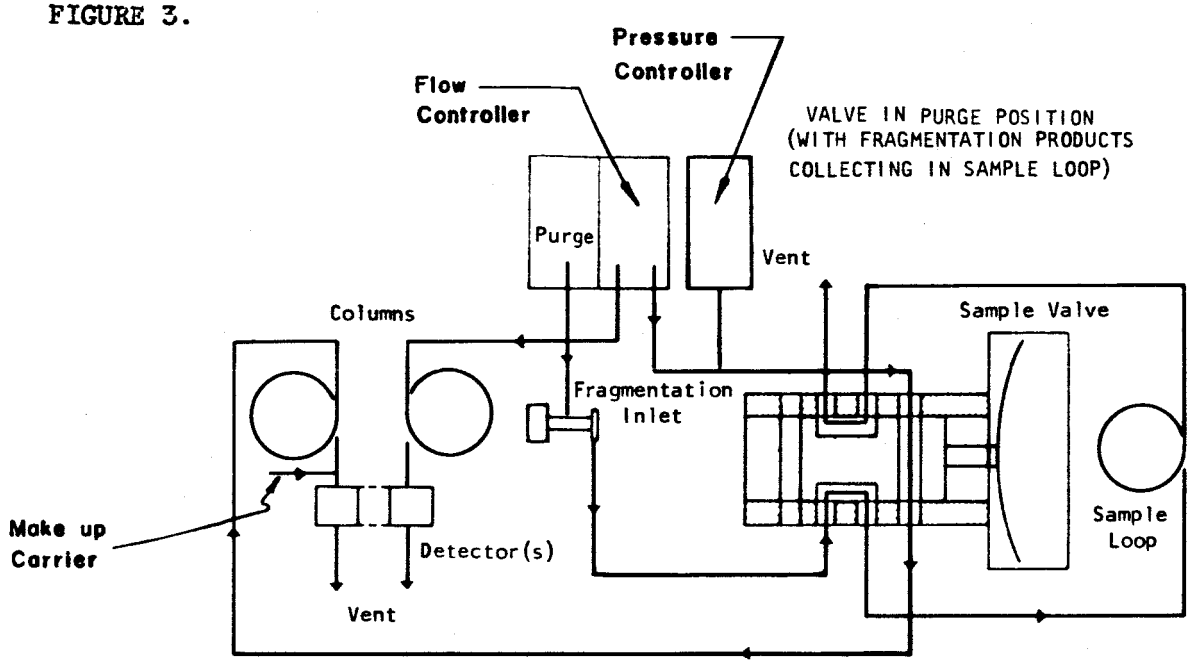
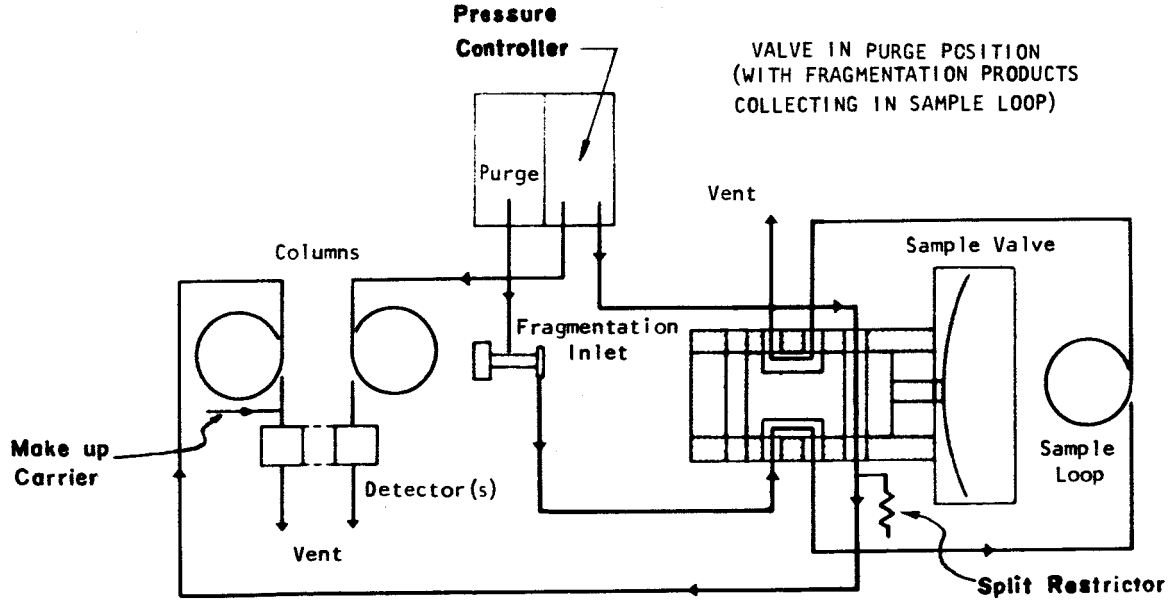


FIGURE 4.



a time delay loop of 10 cc and a restrictor equivalent to 1/39th of the column restriction was installed immediately after the valve to provide a 40:1 split ratio. The 10 cc time delay loop was employed to insure that the entire sample was split before any reached the restrictor, to avoid the possibility of non-linear splitting.

While it is possible that weight limitations may dictate the use of capillary columns for studies on possible life on other planets, there appear to be no advantages and, in fact, there are rather serious disadvantages to their use for earth-based investigations of bacteria.

4. Fragmentation Procedures Employed

4.1. Normal Solids Fragmentation Procedure. The usual procedure for solids fragmentation was employed during the initial and final stages of the project and will be described in the following. The colored end of the sample tube is pressed down on a piece of carbon felt, rough side up, cutting out a piece which just fits the tube, the cutting action is facilitated by rotating the sample tube. One electrode (with gaskets on both sides) is placed in the uncolored side of the sample tube (see figure 5 for orientation of sample tube components). The carbon felt is pushed down with the tool provided or with a glass rod until the carbon felt rests snugly against the electrode. The upstream electrode (containing the platinum wire) is placed in the sample tube. The sample tube is placed in the fragmentation inlet and the discharge is fired for a minimum of 90 seconds to eliminate possible contaminants appearing in the sample fragmentation pattern.

The sample tube is then removed from the inlet and the upstream electrode is removed. A weighed sample is dropped on the carbon felt. The sample is covered with a small amount of silica wool, pressing the wool down onto the sample with the tool or a glass rod. The upstream electrode is then inserted and the sample tube is placed and pneumatically sealed in the fragmentation inlet.

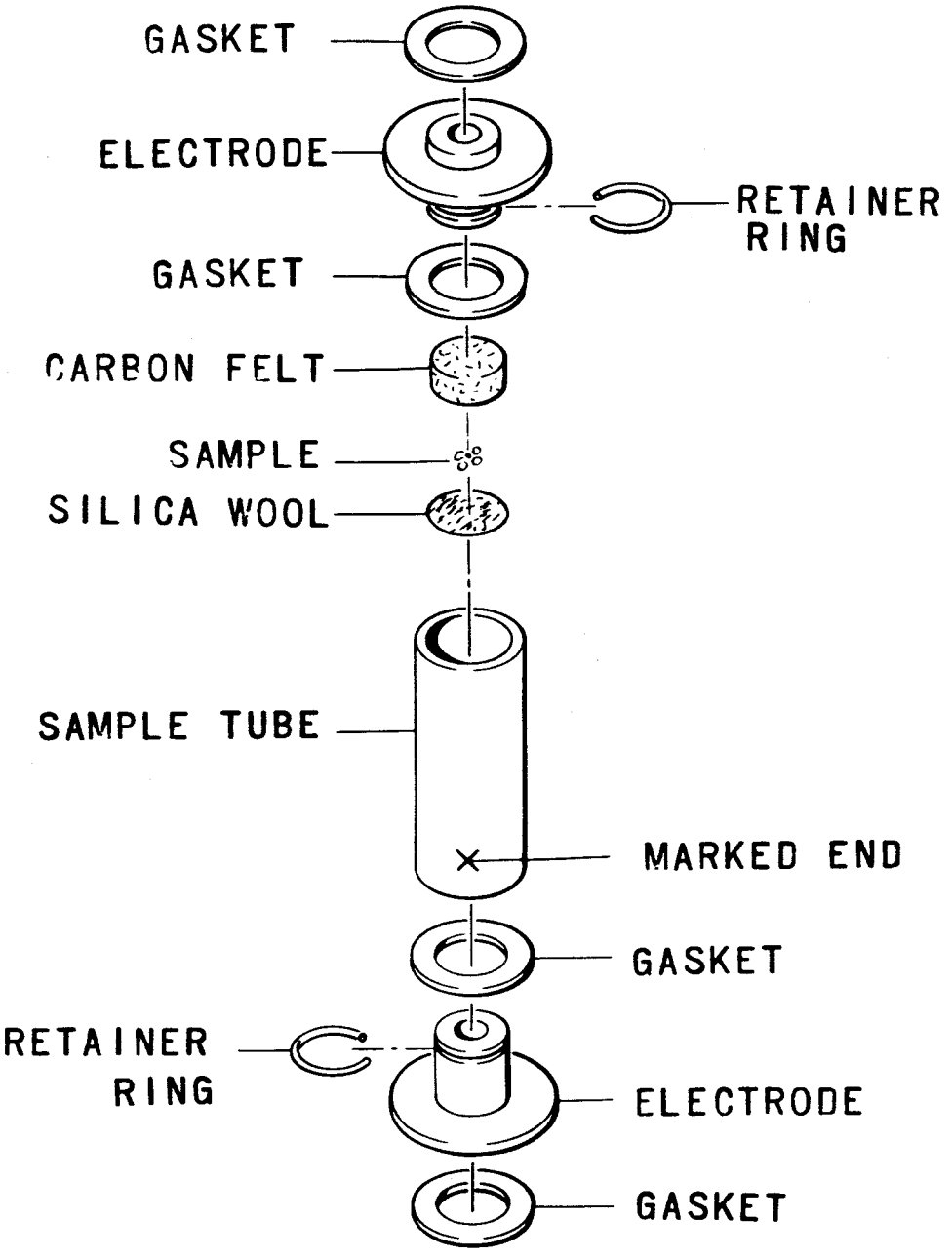


FIGURE 5. SAMPLE TUBE AND COMPONENTS.

A discharge current of one hundred milliamps is normally used for a duration of 30 seconds to fragment the sample. Fifty seconds after the start of fragmentation occurs, the sample loop is switched into the column carrier stream and the program is started. Before this switching is accomplished, the pressure controller is opened to the column carrier stream; approximately one minute after switching, the pressure controller is closed.

In order to achieve the initial temperatures employed, a carbon dioxide cooling accessory was utilized with an automatic function accessory to provide reproducible shut off of the CO_2 cooling unit. This combination proved needed to produce reproducible retention times.

4.2. The Carbon-Carbon Sandwich Procedure. The accepted procedure was found to produce fragmentation patterns which were highly similar for the various bacteria thus providing very little discrimination by means of which the various samples could be identified. After reviewing the work of Garner (1), it was thought that the bacteria patterns obtained by a milder heating of the sample instead of a thorough pyrolysis might be more distinguishable.

For this purpose, lower currents (20 milliamps) were employed; the results, however, were no more distinguishable than the ones obtained using 100 milliamps. The sample was then placed between two carbon felt pieces in an attempt to use the heat generated by the current to produce a milder breakdown. This procedure produced fragmentation patterns that were highly distinguishable, but the reproducibility was extremely poor, probably because of the irreproducible location of the sample between the carbon felt pieces from run to run. Further investigation of this procedure was warranted but prevented by lack of time.

4.3. Restriction of Pre-Fragmentation Time in Heated Zone. In order to avoid the deposition of less volatile fragmentation products within the sample loop, valve, or connecting tubing before the column, it was considered necessary to operate the valve compartment at 250°C. Since the fragmentation inlet is positioned

within the valve compartment, it also is in this high temperature environment. The strikingly greater differences in patterns for different bacteria found by Garner (1) at low pyrolysis temperature as compared to the very minor differences found by Reiner (2), Oyama (3), and in the early runs in this study suggested that loss of sample components in the high temperature environment before fragmentation tended to decrease the distinguishability of the patterns obtained. The procedure was therefore modified by keeping the sample in the Fragmentation Inlet only 30 seconds before firing the discharge. This modification produced distinguishing features that were not previously observed.

Up to this point only helium was utilized as the fragmentation gas atmosphere; since previous results obtained with the fragmentation inlet had indicated that use of other gases could produce pattern changes that were extremely helpful in identification, runs were made with both hydrogen and nitrogen in the fragmentation chamber. At first only 10% of each of these gases in helium was used as the discharge atmosphere, but no significant changes in pattern were observed. Runs were then made in pure hydrogen and pure nitrogen atmospheres to insure adequate gas for reactions that might occur between the discharge gas and the sample, but again no appreciable changes in the patterns were detectable. Helium was therefore again employed throughout the final stages of the investigation.

4.4. Fragmentation Inlet in Room Temperature Surroundings. Since more distinguishable patterns were found when the sample was limited to thirty seconds in the heated zone before fragmentation, it was decided to remove the fragmentation inlet entirely from the heated region of the valve compartment to permit adequate time for purging the sample tube with carrier before fragmentation without heating the sample significantly above room temperature. The fragmentation inlet was accordingly re-mounted on the cover of the valve compartment, with a heating tape placed in the downstream line from the inlet to avoid condensation of the frag-

mentation products on their way to the sample loop; a 110 volt, 400 watt heating tape operated at about 50 volts was found adequate to heat the line to 250°C.

With this modification, it was found possible to obtain nicely distinguishable fragmentation patterns for each of the species of bacteria sampled.

5. Computer Program

With the complex patterns obtained from bacteria fragmentation and the use of the X/(1+X) function on the recorder, it was advantageous to employ a computer program for the tabulation of the peak heights and for the normalization process. Since the patterns are obtained employing temperature programming, which gives nearly constant peak widths, it is acceptable to employ peak heights as a measure of the quantity. The normalization factor used was simply the total millivolts obtained from all of the measurable peak heights. Figure 6 shows the program employed. The variables FLIM, SLIM, and TLIM are the limits between division values where corrections were applied because the recorder did not obey the X/ (1+X) function perfectly. DEL 1, DEL 2 and DEL 3 are the corrections applied to the observed peak height after the appropriate limit, i.e. DEL 1 and DEL 2 applied if a peak height is greater than SLIM. NR is a stop variable. NP and NV are the number of peaks and minima observed on the fragmentation pattern. TF is the time function providing a print out in either seconds or minutes when the retention time is reported in inches. A is the full scale voltage of the recorder and B is used to adjust this if attenuation changes are made.

6. Comparison of Fragmentation Patterns

Some typical fragmentation patterns obtained for bacteria are shown in Figure 7. Table 1 lists the results obtained from duplicate runs of the eleven types of bacteria investigated: relative peak heights in millivolts normalized by dividing by the sum of peak heights for the particular chromatogram are tabulated as functions of observed peak retention times.

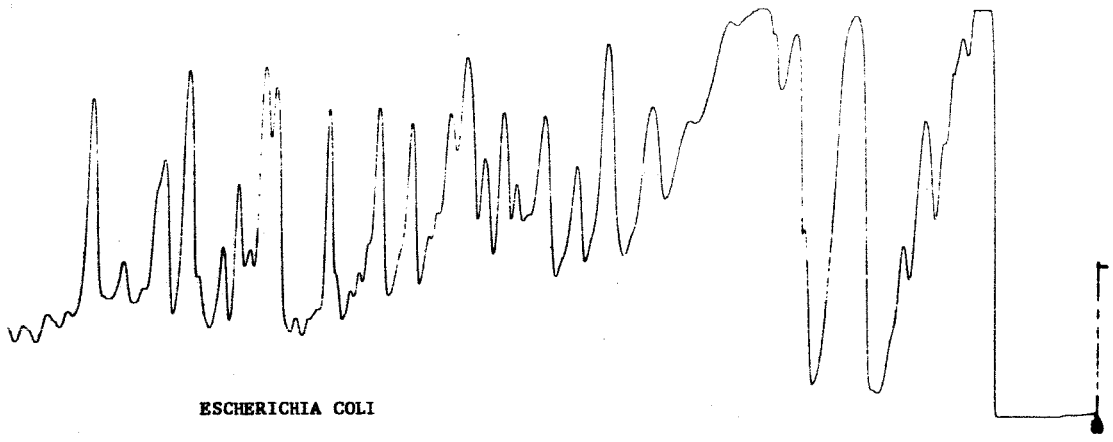
The results were obtained on a 20-foot, 3% Carbowax 20M on Chromosorb W column

Figure 6

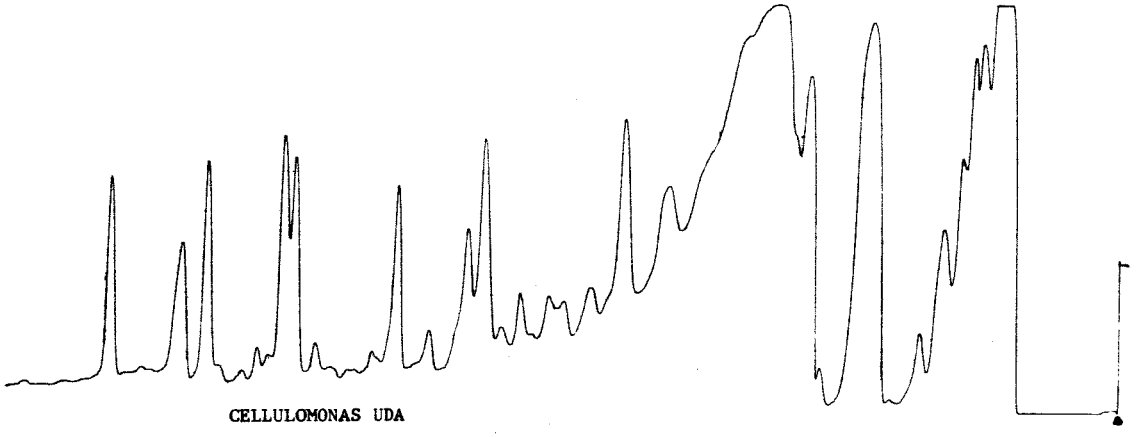
```
C PYROLYSIS CHROMATOGRAM ANALYSIS
      DIMENSION TP(50),TV(30),PK(50),V(30),MS(300),MSG(15)
  100 FORMAT(F10.1,F10.3,F5.2,6A5)
  101 FORMAT (2F10.3,F5.2)
  102 FORMAT(1H1,23H     PYROLYSIS ANALYSIS/15A5//51H PEAK NO.   RET. TI
     1ME   MVOLTS   NORMAL     COMMENT/)
  103 FORMAT(I8,F12.3,F11.4,F9.4,5X,6A5)
  104 FORMAT(/F10.4,17H MILLIVOLTS TOTAL)
  105 FORMAT(6F5.1)
  106 FORMAT(3I5,2F5.1)
  107 FORMAT(15A5)
      READ(1,105)FLIM,DEL1,SLIM,DEL2,TLIM,DEL3
    7 READ(1,106)NR,NP,NV,TF,A
      READ(1,107)(MSG(J),J=1,15)
      K=1
      L=6
      DO 1 I=1,NP
      READ(1,100)TP(I),PK(I),B,(MS(J),J=K,L)
      ADD=0.
      IF(PK(I).LT.FLIM)GO TO 2
      ADD=ADD-DEL1
      IF(PK(I).LT.SLIM)GO TO 2
      ADD=ADD-DEL2
      IF(PK(I).LT.TLIM)GO TO 2
      ADD=ADD-DEL3
    2 PK(I)=PK(I)+ADD
      IF (B .EQ. 0.) B=A
      PK(I)=B*PK(I)/(100.-PK(I))
      K=K+6
    1 L=L+6
      DO 3 I=1,NV
      READ(1,101)TV(I),V(I),B
      ADD=0.
      IF(V(I).LT.FLIM)GO TO 4
      ADD=ADD-DEL1
      IF(V(I).LT.SLIM)GO TO 4
      ADD=ADD-DEL2
      IF(V(I).LT.TLIM)GO TO 4
      ADD=ADD-DEL3
    4 V(I)=V(I)+ADD
      IF (B .EQ. 0.) B=A
    3 V(I)=B*V(I)/(100.-V(I))
      SUM=0.
      M=0
      DO 5 I=1,NP
      IF(TP(I).GT.TV(M+1))M=M+1
```

Figure 6 con't.

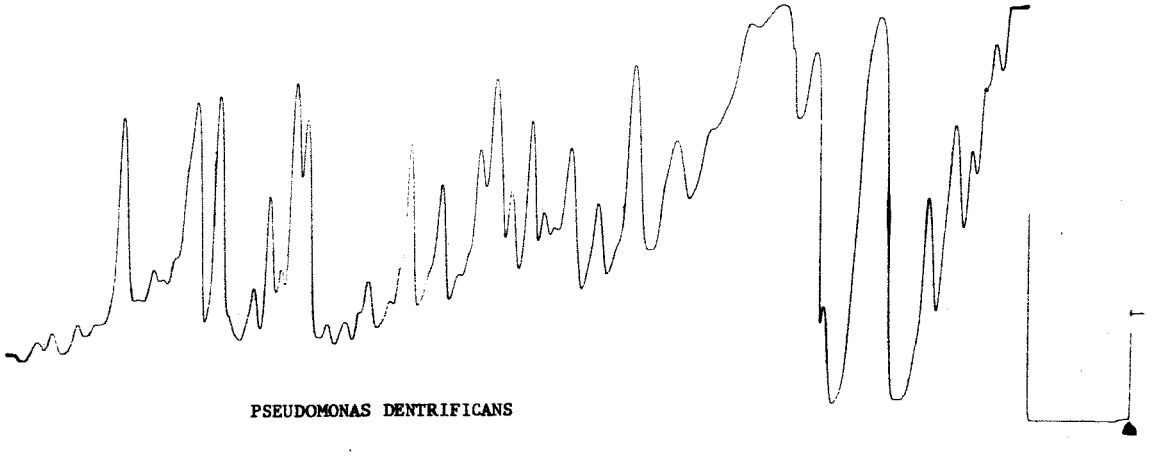
```
      DBASE=V(M)-V(M+1)
      DTB=TV(M+1)-TV(M)
      DTP=TV(M+1)-TP(I)
      PK(I) = PK(I) - V(M+1) - (DTP*DBASE/DTB)
    5 SUM=SUM+PK(I)
      K=1
      L=6
      WRITE(3,102)(MSG(J),J=1,15)
      DO 6 N=1,NP
      T=TP(N)*TF
      PNORM=PK(N)/SUM
      WRITE(3,103)N,T,PK(N),PNORM,(MS(M),M=K,L)
      K=K+6
    6 L=L+6
      WRITE(3,104)SUM
      IF(NR.EQ.100)STOP
      GO TO 7
      END
```



ESCHERICHIA COLI



CELLULOMONAS UDA



PSEUDOMONAS DENTRIFICANS

Figure 7.

TABLE 1

Retention Time in Min.	Pseudomonas denitrificans		Photobacterium fischeria		Escherichia coli		Azobacter vinelandii		Streptomyces achromogenes		Serratia marcescens		Bacillus subtilis		Clostridum pasteurianum		Cellulomonas uda		Corynebacterium insidiosum		Rhodospirillum rubrum	
3.79	.0186^{S}	.0128^{S}	.0091^{S}	.0054^{S}							.0129^{S}	.0301^{S}	.0071^{S}	.0019^{S}			.0280^{S}	.0372^{S}				
4.23	.0555^{S}	.0396^{S}	.0203^{S}	.0149^{S}	.0275^{S}	.0346^{S}	.0966^{S}	.0514^{S}	.0235^{S}	.0300^{S}	.0672^{S}	.0636^{S}	.0207^{S}	.0248^{S}	.0501^{S}	.136^{S}	.0309^{S}	.0409^{S}	.0127^{S}	.0257^{S}	.0434^{S}	.0450^{S}
10.94	.0422	.0301	.0428	.0297	.0428	.0367	.0168	.0129	.0291	.0391	.0219	.0260	.0363	.0377	.0553	.0609	.0316	.0514	.0228^{D}	.0213^{D}	.0788	.0792
12.03	.130	.112	.140	.0951	.124	.147	.0972	.0721	.136	.122	.0592	.0923	.1277	.1393	.1414	.2810	.139	.196	.0913	.115	.141	.137
12.78	.0145	.0111	.0129	.0153	.0229	.0177	.0230	.0263	.0102	.0126	.0447	.0087	.0121	.0135	.0119	.0105	.0105	.0170	.0114	.0102	.0151	.0128
13.54	.0205	.0306	.0366	.0516	.0360	.0399	.0039	.0045	.0254	.0064	.0031^{D}	.0036^{D}	.0061	.0087	.0067	.0078	.0098	.0079	.187	.220	.0303	.0106
13.99							.0009	.0013					.0024	.0123			.0124	.0045				
14.23	.0133	.0114	.0131^{S}	.0102^{S}	.0121	.0141			.0130	.0039									.0163^{S}	.0205^{S}	.0104	.0143
14.47			.0092	.0136																		
14.60	.0365^{D}	.0460^{D}	.0206	.0179	.0352	.0429	.0348	.0274	.0159	.0129	.0062^{D}	.0060^{D}	.0042	.0154	.0106	.0207	.0147	.0063	.0163	.0265	.0202	.0221
15.01	.0172	.0167	.0099	.0079	.0161	.0218	.0023	.0013	.0057	.0060	.0043	.0029	.0048	.0071	.0075	.0086	.0058	.0033	.0074	.0138	.0066	.0067
15.44	.0858	.0843	.129	.104	.105	.101	.0684	.0726	.0767	.0843	.0592	.0841	.120	.110	.118	.113	.117	.126	.0867	.0938	.105	.128
15.92	.0424	.0318	.0444^{S}	.0278^{S}	.0315^{S}	.0426^{S}	.0076	.0079	.0270	.0327	.0247	.0412	.0313	.0295	.0504	.0475	.0440^{S}	.0453^{S}	.0214	.0401	.0463	.0451
16.22			.0414^{S}	.0238^{S}															.0154	.0253		
16.39							.0016	.0011													.0050	.0019
16.92	.0208	.0200	.0077	.0234	.0352	.0376	.0582	.0927	.0100	.0074	.0149	.0091	.0117^{D}	.0111^{D}	.0103	.0210	.0091	.0068	.0103	.0141	.0266	.0214
17.67	.0281	.0344	.0441	.0737	.0634	.0474	.159	.185	.0262	.0535	.0500	.0451	.0511	.0474	.0635	.0380	.0711	.0718	.0704	.0331	.0594	.0718
17.92							.0088	.0071					.0184^{S}	.0155^{S}								
18.23	.0019	.0026	.0082	.0092	.0032^{S}	.0043^{S}							.0119^{S}	.0108^{S}			.0057	.0098	.0039	.0045		
18.71	.0028^{D}	.0052^{D}					.0019	.0001	.0017	.0023			.0078^{S}	.0073^{S}	.0016	.0017	.0019	.0052	.0012	.0023	.0036^{S}	.0027^{S}
18.93					.0155	.0465	.0191	.0090			.0556	.0535			.0029	.0037	.0019	.0052			.0119	.0099
19.07																					.0041	.0033
19.28	.0010	.0015	.0017^{D}	.0014^{D}					.0021^{D}	.0010^{D}			.0037^{S}	.0029^{S}			.0026	.0035	.0006^{D}	.0010^{D}		
19.70	.0011	.0014	.0038	.0043	.0012	.0012	.0072	.0077	.0033	.0025	.0052	.0029	.0035^{S}	.0017^{S}	.0049	.0078	.0078	.0058	.0016	.0028	.0014	.0012
20.24	.0421	.0485	.0825	.0986	.0751	.0620	.118	.158	.0043	.0537	.0639	.0567	.0814	.0707	.0589	.0596	.0967	.0952	.0829	.0501	.0742	.0834
20.49	.0884	.0787	.0796	.0878	.100^{S}	.0892^{S}	.0200	.0200	.0982	.0756	.0504	.0446	.0969	.116	.0452	.0712	.123	.0896	.0666	.0651	.0764	.0713
20.86	.0047	.0072			.0065^{S}	.0076^{S}	.0230	.0092			.0088	.0052			.0026	.0035	.0053	.0027	.0033	.0052	.0091	.0092
21.12	.0158	.0187	.0049	.0146	.0168^{S}	.0177^{S}			.0109	.0033			.0066	.0134			.0067	.0016	.0071	.0113	.0085	.0068
21.57	.0036	.0048	.0027^{D}	.0072^{D}	.0069	.0078	.0007	.0005	.0022^{D}	.0007^{D}	.0058	.0026	.0051^{D}	.0064^{D}	.0096	.0018	.0021	.0003	.0020^{D}	.0031^{D}	.0037	.0027
22.08							.0036	.0029														
22.36	.0439	.0634	.0928	.115	.117	.0841	.0301	.0318	.0737	.0598	.0706	.0732	.0571	.0482	.0635	.0245	.0920	.0781	.106	.0538	.0743	.0854
22.94	.0417	.0577	.0191^{D}	.0359^{D}	.0168^{D}	.0231^{D}	.0018	.0031	.0704^{D}	.0106^{D}	.0173^{D}	.0097^{D}					.0404	.0045	.0672	.0894	.0084	.0058
23.14							.0018	.0026					.0044	.0036	.0480^{D}	.0041^{D}					.0092	.0070
23.45																					.0017	.0012
23.73	.0030	.0054	.0039^{D}	.0092^{D}																		
23.91	.0035	.0067			.0052	.0052			.0030	.0019	.0085^{D}	.0082^{D}	.0019	.0007	.0674^{D}	.0039^{D}	.0030^{D}	.0020^{D}	.0044^{D}	.0064^{D}	.0049	.0032
24.32							.0088	.0044													.0038^{S}	.0023^{S}
24.72	.0251	.0483	.0855	.0781	.0650	.0506	.0880	.107	.0390	.0417	.0834	.0972	.0652	.0340	.125	.0225	.0076	.0698	.0813	.0284	.0674	.0782
25.81			.0015	.0029							.0051	.0041	.0008	.0005							.0034	.0021
Normalization Factor	30.87	47.30	18.60	71.01	40.05	56.79	30.91	25.82	14.41	16.61	27.40	8.107	40.29	44.12	10.37	4.642	15.93	10.33	27.28	29.59	60.80	44.70
Total Millivolts																						

held isothermal at 50°C for four minutes, then programmed to 200°C at 7.5°C/min. and finally left isothermal at 200°C for approximately ten minutes. Helium flow rates were 5 cc/min. through the fragmentation inlet and 25 cc/min. through the column. The discharge was fired for 30 sec. at 100 mamps; the inlet was mounted on top of the valve compartment cover for these runs.

It can readily be observed that significant differences in the peak patterns do occur, superimposed upon a structure of highly similar peaks for all of the bacteria. For example, the peak at 19.28 minutes appears in only six of the eleven bacteria. The peak at 22.08 minutes appears only in the case of Azobactor vinelandii. The presence of such unique peaks, as well as changes in peak height in the commonly occurring peaks, offer considerable information for the characterization of the bacteria. Retention times of component peaks are found to be reproducible to within 1%, which permits positive identification of a peak even in a pattern as complex as those obtained for the bacteria.

7. Mesoporphyrin IX and Gelatin vs. Bacterial Patterns

Table II shows comparison runs of bacteria, gelatin, and mesoporphyrin IX. The species of bacteria, Serratia marcescens, chosen was known to contain porphyrin derivatives. The patterns obtained however, as indicated by the table, demonstrate a stricking resemblance between bacteria and protein patterns, while the fragmentation pattern of mesoporphyrin IX exhibits very little resemblence to either. The results in Table II have been normalized by the total millivolts observed in the fragmentation patterns. The patterns were obtained on a 20-foot 3% SE 30 column on Chromosorb W, run isothermally at 0°C for four minutes, then programmed at 12.5°C/min. to 250°C.

It appears that all protein-containing materials display considerable similarity of fragmentation pattern, and provide patterns far different from those obtained in non-protein substances. This fact in itself is probably the strongest argument in favor of fragmentation chromatography as a life-detection tool.

TABLE 2

BACTERIA		KNOXGELATIN		MESOPARPHYSIN	
I Retention Time in Min.	II Peak Height Total Peak Heights	III Retention Time in Min.	IV Peak Height Total Peak Heights	V Retention Time in Min.	VI Peak Height Total Peak Heights
1.50	.74	1.50	.73	1.46	.70
1.80	.039	1.81	.029		
				1.94	.077
2.15	.14	2.12	.16		
2.70	.0062	2.70	.0048		
	.0044		.0044		
3.00	.0015	3.00	.0039		
				9.88	.022
10.35	.024	10.35	.018		
12.50		12.50	.023		
12.85	.018	12.82	.011		
14.45	.00071	14.45	.0012		
	.00064		.0011		
	.0022		.0021		
	.0018		.0016		
15.50	.0033	15.50	.0019		
				15.70	.028
17.00	.0027	17.00	.0030	17.14	.012
				17.60	.048
18.20	.0036	18.20	.0027		
				18.66	.034
19.45	.0018	19.45	.0013		
19.95	.0012	19.95	.0015	19.98	.030
20.56	.0040	20.53	.0031		
24.25	.0020	24.25	.0017		

8. Suggestions for Further Work

Many parameters concerning fragmentation of life-related materials employing either electrical discharge or thermal fragmentation have yet to be investigated and are cited here as suggestions for possible future work.

Two operational parameters which should be studied in a further application of the discharge fragmentation technique are discharge gas flow and sample size. When the fragmentation inlet was removed from the valve compartment, it was noted that sample vapor diffuses back into the discharge, producing a bright blue color during the initial stages of fragmentation. This situation can lead to destruction of some portion of the highly characteristic most volatile portion of the fragmentation pattern. A larger helium flow through the discharge, e.g., 15 cc/min., should be employed for only these initial seconds of fragmentation with the flow switched back to the normal 5 cc/min. after this initial stage. This extra flow should prevent sample from backing up into the discharge zone while only slightly diminishing the sample collection efficiency of the sample loop since the higher flow would be employed for only a very few seconds. Probably the most important further work with the fragmentation inlet should be the investigation of the effect of sample size on the fragmentation pattern. In this study samples were weighed to a tenth of a milligram and repeated samples were held only to three tenths of a milligram on an average sample size of 1.3 milligrams. This probably hurt the reproducibility of peak heights in the patterns obtained. Reiner (2)(2a) reported controlling sample weights to one microgram, and reproducing, at least on the one duplicated run he exhibited, to five micrograms on an average sample of one half a milligram. The remarkable reproducibility of his runs are at least in some portion due to this controlling of sample size. Unfortunately, his resolution was poor and slight changes in heights may have been missed. If varying sample size changes the fragmentation pattern, it may be necessary to produce standard patterns at several sample sizes to insure a positive identification of the unknown bacteria. The ability to detect bacteria in exobiological applications

should not be hampered by peak height irreproducibility, since the unique qualitative behavior of all bacteria and protein patterns is sufficient to recognize a life-related substance. An aspect of bacterial identification that was not investigated is the function played by the growth medium. One may obtain differing patterns from identical bacteria if a different culture media were employed.

Throughout this study lyophilized bacteria were employed; however, whole bacteria may not only produce different fragmentation patterns but they may indeed produce more distinguishable patterns, since it has been shown that the relatively volatile portion contains the most significant differences.

Of major importance to fundamental bacterial investigations is the investigation of the fragmentation patterns of amino acids, low molecular weight polypeptides, proteins, of known structure and other life-related compounds in an effort to determine the origin and identity of the peaks that have consistently appeared in all bacterial fragmentation patterns. With the fragmentation inlet, introduction of an injection port in place of the sample tubes facilitates measurement of retention volumes for reference components, so that fragment peaks can be assigned retention index values on different columns and can be compared with desired reference materials.

When a standard procedure for the fragmentation of bacteria has been adopted, it would be highly desirable to compile a reference library of fragmentation chromatograms of as many known bacteria as possible.

9. Summary

The characterization of bacterial species has long involved highly-trained personnel applying considerable time and effort. The determination of bacteria through fragmentation gas liquid chromatography would diminish the time and effort required for a positive identification. The speed factor should play an important role in the diagnosis and treatment of illnesses. The investigation for life on planets other than the earth is an extremely interesting problem which requires,

a completely automated rapid system. Both of these requirements are met by fragmentation chromatography. 27756

In this study an electrical discharge fragmentation device has been successfully applied to the determination of fragmentation patterns for eleven species of bacteria. Operating parameters studied include the effects of discharge current, discharge gas composition and the choice of chromatographic column on the patterns obtained. Procedures were developed for obtaining highly reproducible retention times. It was found that the greatest differences in patterns for different bacteria occur in fragments which can be lost even under conditions of mild heating (to 250°C) and purging before fragmentation, the instrument and procedure were accordingly modified to prevent this loss, and distinguishable patterns were then obtained for the eleven species of bacteria studied. It has been found that approximately 10 peaks can be labelled as steming from protein substances.

Author

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