230

THE ISOLATION AND IDENTIFICATION OF A NATURAL FLY ATTRACTANT

by

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Table of Contents

List of Tables

List of Figures

List Of Spectra

Justification

Flies, which transmit dozens of plant, animal and human diseases, are considered one of our greatest public health hazards. Because of recent attitudes against the use of pesticides, it is now necessary to find a new way to control fly populations. It is estimated that there are 20,000 species of flies in the North American continent and 90,000 species world-wide. (Scott and Littig, 1960). If a way could be found to break their life cycle by natural means, then a drastic reduction in fly population might be achieved.

Two major natural methods have been employed thus far. One of these is the use of beetles to bury manure pats in which many species of flies lay their eggs. The second is by use of a compound such as muscalure, (z)-9-tricasene, a sex attractant liberated by the female house fly. However, this attracts only the males and the males that are not trapped can still fertilize most of the remaining females.

Due to the concentration of cattle on the prairie ranges of the United States, and other countries, the fly population problem is becoming most intense. Face and horn flies are our greatest range pests. What is needed, then, is a way to

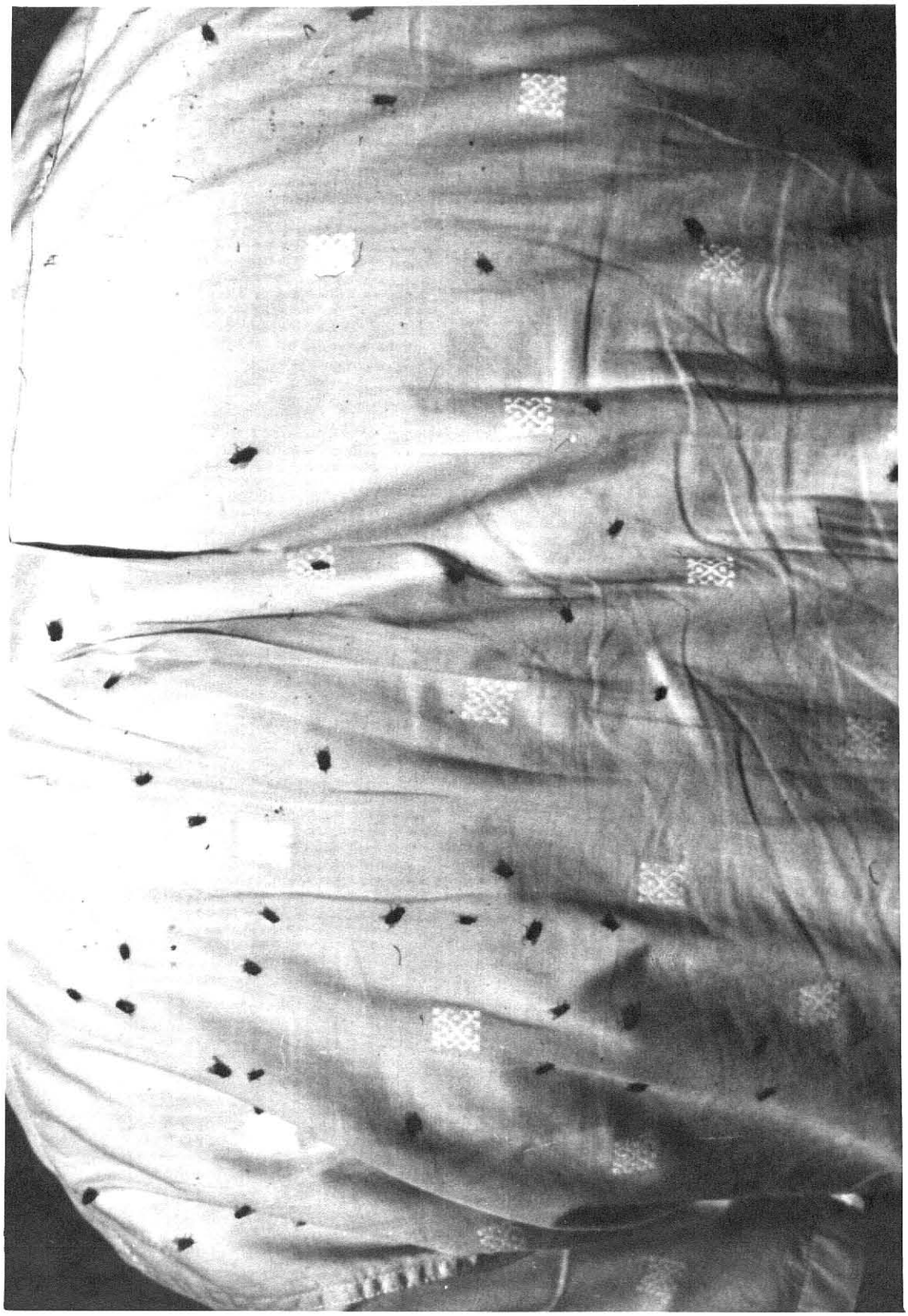
attract the female flies, so that they can be trapped and removed from the environment without causing environmental pollution by widespread spraying. Years ago, Dr. C.W. Pitts, of the KSU Entomology Department, found that animal manure is a part of the life cycle of several species of flies, in that this is where the female layed her eggs. He also observed that there appeared to be a chemical or combination of chemicals that served as a natural attractant, as shown in photo 3, page 12. The purpose of this research was to find and identify this natural attractant. This has been done. It is meta-xylene and has been found to be present in cow, horse, sheep, goat and chicken manure, as well as being found in garbage. This compound has been tested effectively with both face flies and house flies, and has been determined to have its greatest attraction when present in a concentration on the order of 5 to 15 parts per million.

Introduction

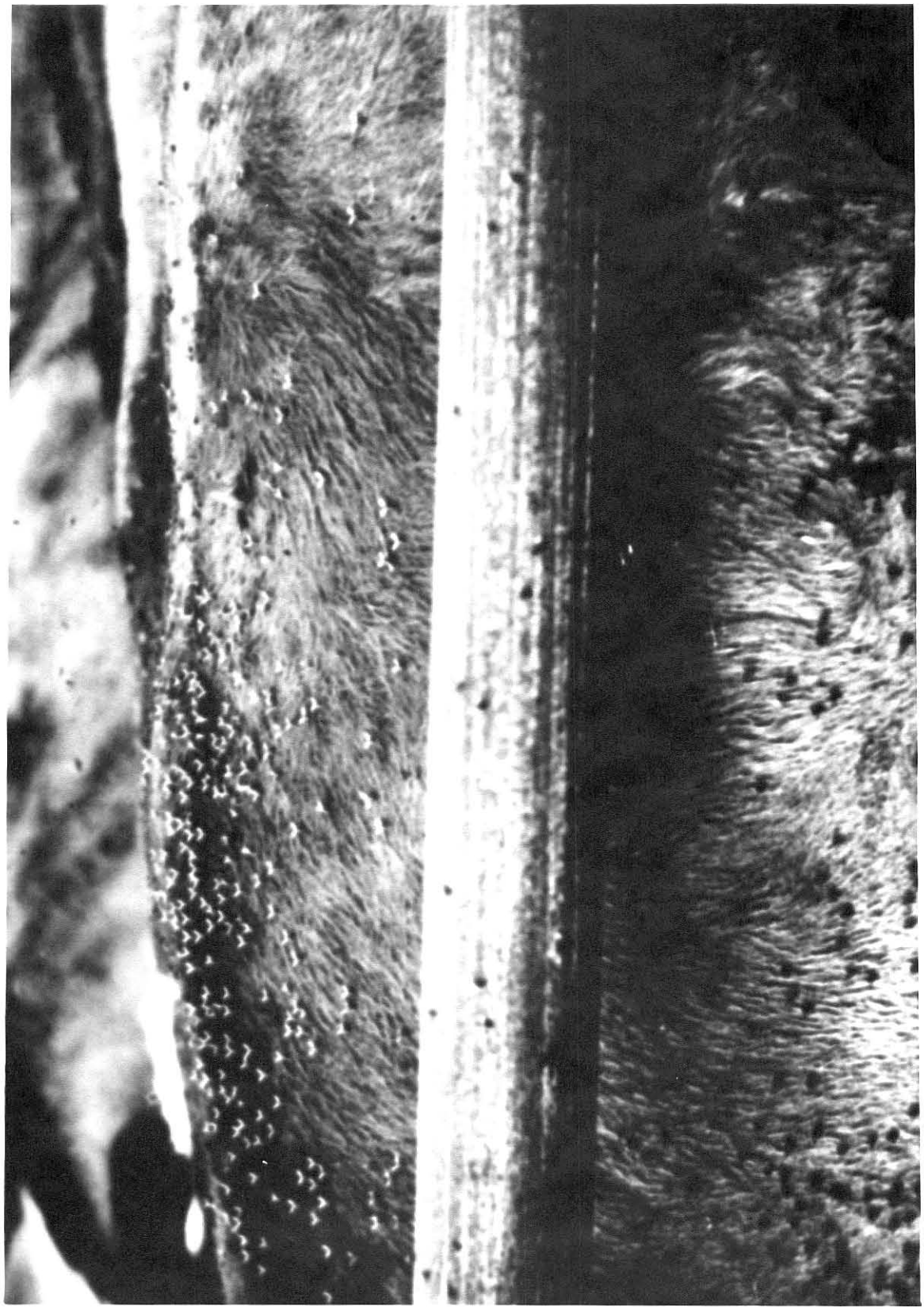
Flies have been the intimate companions of man since long before the dawn of recorded history. Year after year, they have annoyed him, and have plagued him with vicious bites. Fly larvae have infested the flesh of man and his domestic animals, and have attacked, and destroyed man's crops. More important, flies have carried disease (typhoid, dysentery, diarrhea, African sleeping sickness, onchoerciasis, and many others) and death to millions of people the world over. Today it is recognized that flies constitute one of the greatest of public health hazards, and that abatement of fly populations is essential to the control of many serious and widespread diseases.

An example of the extent to which a fly population can cause problems is that of Australia. Some areas of this continent have a very intensive fly problem, as do certain areas of Latin America. In these areas, it is necessary to wear hats with strings hanging from the brim, in order that, when one shakes his head, the strings are set in motion and cause the flies to move away for a short time. This is only a temporary deterent, however, and the flies soon return. The photographs on pages 4 and 5 show the strong concentration of flies,

Photograph one; flies on the back of an Australian man, illustrating the high concentration of flies in this country.



Photograph two; flies on the back of a cow in Australia.



both on a human and on the back and sides of an animal. Dr. G.F. Bornemissza of the Commonwealth Scientific and Industrial Research Organization, first put forward the idea of introducing dung beetles into Australia in a scientific paper published in 1960 (Bornemissza, 1960) and in 1963 the present program came under his leadership. Indigenous beetles do bury the droppings of the native marsupials, but they cannot cope with the large, wet, dung pats of domestic stock introduced by European Man. The dung beetles are able to bury the manure pats underground, thus reducing the availability of the medium in which the flies hatch their eggs. Dung beetles cannot always prevent the insects from laying their eggs in the fresh droppings, but if they bury these droppings before the flies have completed their development, then they will effect control. The two major Australian pests which breed in cattle dung are the bushfly and the buffalo fly. (Bornemissza, 1960).

The introduction of the African dung beetle has proven successful when the climate is right but now one wonders if the beetles themselves will be a problem.

One can identify a number of points with respect to flies in relation to human welfare. Some of these points are as follows.

Annoyance; domestic flies can be a serious threat to human efficiency. Biting flies disrupt picnics and other recreational activities as well as the pioneering efforts of mankind. In Canada, for instance, large areas of fertile land remain unsettled, due in large part to the presence of annoying and biting flies.

Bites; not all flies bite, but those which do can cause serious trouble. Biting flies do not have venom in the usual sense. Instead, the effects of their bites are the result of a reaction to the saliva poured into the wound to prevent clotting during the feeding process. The stable fly is common around human habitations and its bite can be quite severe.

Myiasis; many species of flies are capable of laying eggs or larvae on the flesh of mammals and other animals. The larvae thus deposited can invade the flesh of the host animal producing a condition known as myiasis. - Wild animals, such as deer and rabbits, are commonly afflicted, as are many domestic animals, especially cattle and sheep. Human myiasis, while not common, occurs in all parts of the United States as well as in most other countries.

Mechanical transmission of disease; flies carry disease-causing organisms in five ways: (1) on their mouth parts,

(2) through their vomitus, (3) on their body and leg hairs, (4) on the sticky pads of their feet, and (5) through the intestinal tract by means of fly feces.

Biological transmission of disease; many flies, particularly biting flies, are involved in the biological transmission of some of the most serious and commonest of vector-borne diseases such as African sleeping sickness and leishmaniases.

Agricultural importance; many species of flies attack and damage plants directly (Hessian fly, cabbage maggot, onion maggot, apple maggot, clover seed midge, seed corn maggot, and others). Some flies transmit plant diseases (blackleg of cabbage, bacterial soft rot of vegetables, etc.). In addition, flies annoy, cause myiasis in, and transmit diseases to domestic animals. (Scott and Littig, 1960).

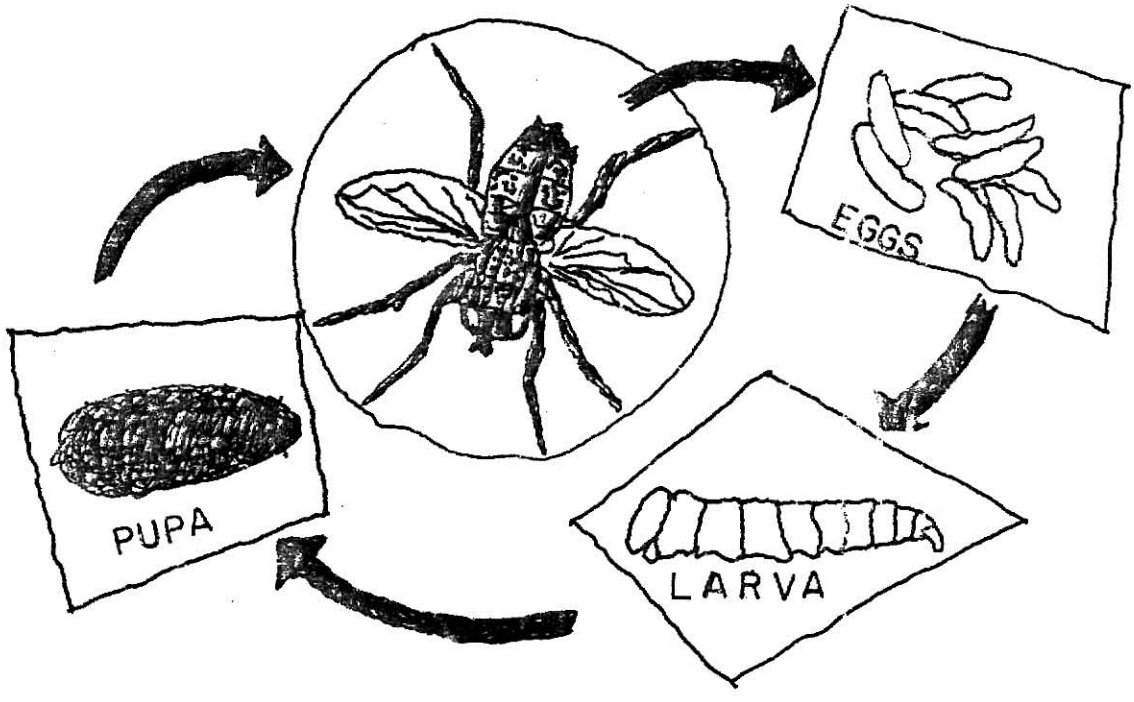
The work done in this project was performed using face flies. The habitat of the face fly and house fly is very nearly the same, or can be under certain conditions. The face fly is a blood-sucking fly whereas the house fly is not to such as great an extent.

Almost any type of moist, warm organic material may furnish suitable nourishment for fly larvae of these two types.

Animal manure is an excellent breeding medium, accounting for as many as 95% of the house flies in some rural areas. This medium accounts for nearly 100% of the face fly breeding areas. Fresh horse manure may produce as many as 1,200 larvae per pound. Manure from other animals (cows, pigs, rabbits, fowl, etc.) is also very suitable. Accumulations of fowl excrement are commonly infested with larvae, but scattered droppings in dry pens are seldom infested. In the laboratory, face flies are distinguished from house flies only by minute characteristics, (Sabrosky, 1959). However, the habits of these two flies are so different, they can be readily distinguished in the field.

Dr. Charles W. Pitts, Professor in the Entomology Department at Kansas State University, and others, have shown that animal manure is a necessary part of the life cycle of the face fly and unless a female has fresh manure available when she is ready to lay her eggs, she will become egg-bound and die. This medium is also vital to the hatching and nutrition of the larvae of the face fly. The life cycle of the face fly is shown diagrammatically on page 10. The life cycle of the common house fly is similar but the animal manure is not a necessity, since the house fly can hatch in other organic waste as well.

Figure one; The life history of the fly

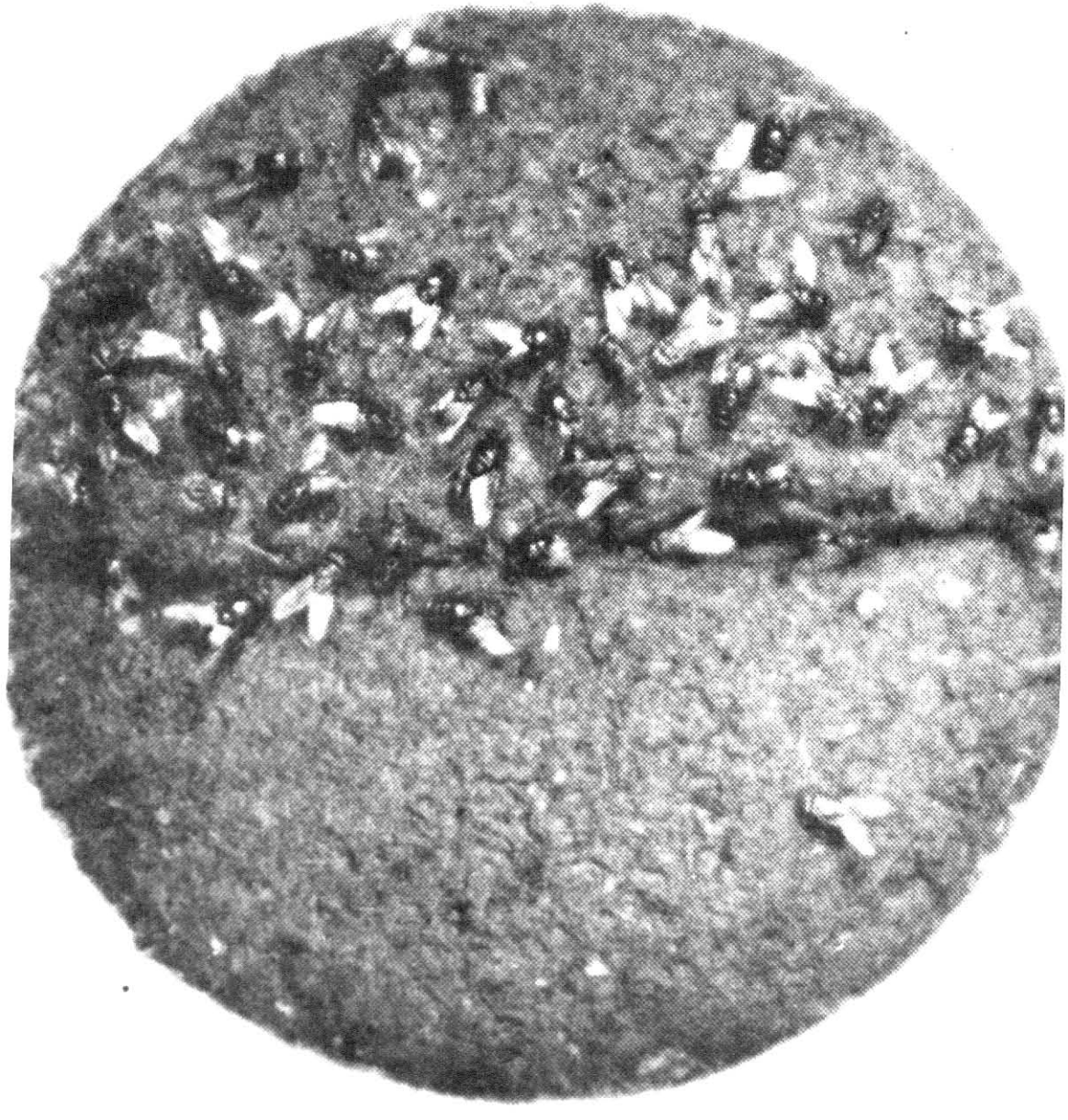


LIFE HISTORY OF THE FLY

It has been shown, in various studies done in the Entomology Department at Kansas State University, that cow manure has in its composition, a material which is a natural fly attractant. This material serves to inform the fly of the presence of the fresh manure, the female fly then landing on the manure and depositing her eggs. The eggs, once laid on the surface of the fresh manure, burrow under the surface and the larvae, which are hatched from the eggs, receive their nourishment from the manure until ready to be advanced to the next stage in the life cycle. It has been demonstrated, by Dr. Pitts, that cattle who are on a diet consisting mainly of hay, produce manure which has a very great capacity for the attraction of flies.

The photograph on page 12 shows a patty of manure collected from a bull on a high-hay diet. This was lyofalyzed, and one-half of the manure reconstituted with the liquid obtained from the lyofalyzation process. The other half was reconstituted with distilled water. This patty was then placed in a cage of female flies who were known to be ready to lay their eggs. This time can be determined within very close limits by rearing the flies in a laboratory environment, with close control of the number of days after hatching of the adult flies. One can see the strong concentration of flies on one half of the patty and very few on the other half. The one

Photograph three; The difference in fly attraction to a cow manure sample which has been lyofalyzed and subsequently reconstituted, one-half with attractant containing liquid and the other one-half with distilled water.



half does not have the attractant material present in its composition, that which was reconstituted with distilled water. This type of reconstitution has been repeated many times, and indicates that the attractant material is volatile.

The aim of this work is to attempt to determine whether or not the attractant material in animal manure, and other sources, is a single compound, or a mixture, and to identify its composition if possible. It is with this problem that the remainder of this presentation will be concerned.

The basic procedure was to adsorb the vapors on a compound, Tenax-GC, then to separate them by gas chromatography. Various fractions were collected and tested for effectiveness using the Bey olfactometer shown in figure 3, page 25. The active compound was then trapped and its composition determined by mass spectrometry and nuclear magnetic resonance spectroscopy. The suspected compound was then compared in the gas chromatograph with the active compound and they had equal retention times. Finally the compound was tested with the female face and house flies and found to be active.

Column Packing Material

The columns used in the gas chromatograph for the early work done on this project were made of a 20% carbowax 20M liquid phase on Chromasorb-P, DMCS treated, acid washed, solid support obtained from Analabs, 80 Republic Drive, North Haven, Conn. The columns were conditioned in the gas chromatographic oven at 175°C for twenty-four hours. The separation accomplished by use of these columns was very good, but the maximum temperature at which they could be utilized was on the order of 200°C. It was thought that the temperature necessary was perhaps higher than this so a new packing material was sought that would withstand higher operating temperatures. The early columns used were glass, 10 feet in length and had 3/16 inches outside diameter.

The columns used in the later portion of this project were packed with a new, porous polymer manufactured by AKZO Laboratories and marketed by Enka N.V. of the Netherlands through Applied Science Laboratories, Inc., P.O. Box 440, State College, Penn. This packing material is based on a polymer of 2,6-diphenyl-p-phenylene oxide and is sold as Tenax-GC. It is suitable for the separation of high-boiling polar compounds such as alcohols, polyethylene glycol compounds,

diols, phenols, mono and di-amines, ethanolamines, amides, aldehydes, and ketones. It has the advantages of high maximum operating temperatures, (375°C), short retention times, stable baseline after short conditioning times, relatively low temperature for effective separation and short column lengths required. Conditioning of the columns is achieved by passing carrier gas through the column at a rate of about 15 ml/min for 1 hour at room temperature. Without interrupting the gas flow, the oven is heated at a programmed rate of 6° to 8° per minute from room temperature to 20°C above the maximum operating temperature, but not above 350°C, as a safe limit, without damage to the packing material. The column is kept at this temperature for at least 1 hour, then allowed to cool to the desired starting temperature. The column should then be ready for use. Repeat this procedure if necessary. See technical bulletin No. 24, by Applied Science Labs. The columns used with this packing material were two feet in length.

Apparatus

A Bendix model 2200 gas chromatograph equipped with a flame ionization detector and programmable temperature capability was used throughout this work. A Bristol Dynamaster recorder equipped with a pressure pen and a chart speed of one-half inches per minute was used to record all chromatograms.

All columns used in the gas chromatographic oven were of glass, 3/16 inch outside diameter, two feet long, for the later work. The earlier columns used were of glass, 3/16 inches outside diameter, 10 feet long.

Blenders used were manufactured by Waring Products Division, Dynamics Corporation of America, New Hartford, Conn.

The air pump used was a Neptune Dynapump, model 4K. This may be obtained from a number of supply sources.

Special items required for this project which had to be fabricated from metal were made by Mr. Al Nielson, Metal Shop, Chemistry Dept., KSU. The specialty glass items were made by Mr. M. Ohno, Glassworking Shop, Cardwell Hall, KSU.

AEI MS-9 Double Focusing Mass Spectrometer.

Varian XL100-15 Nuclear Magnetic Resonance Spectrometer.

Experimental

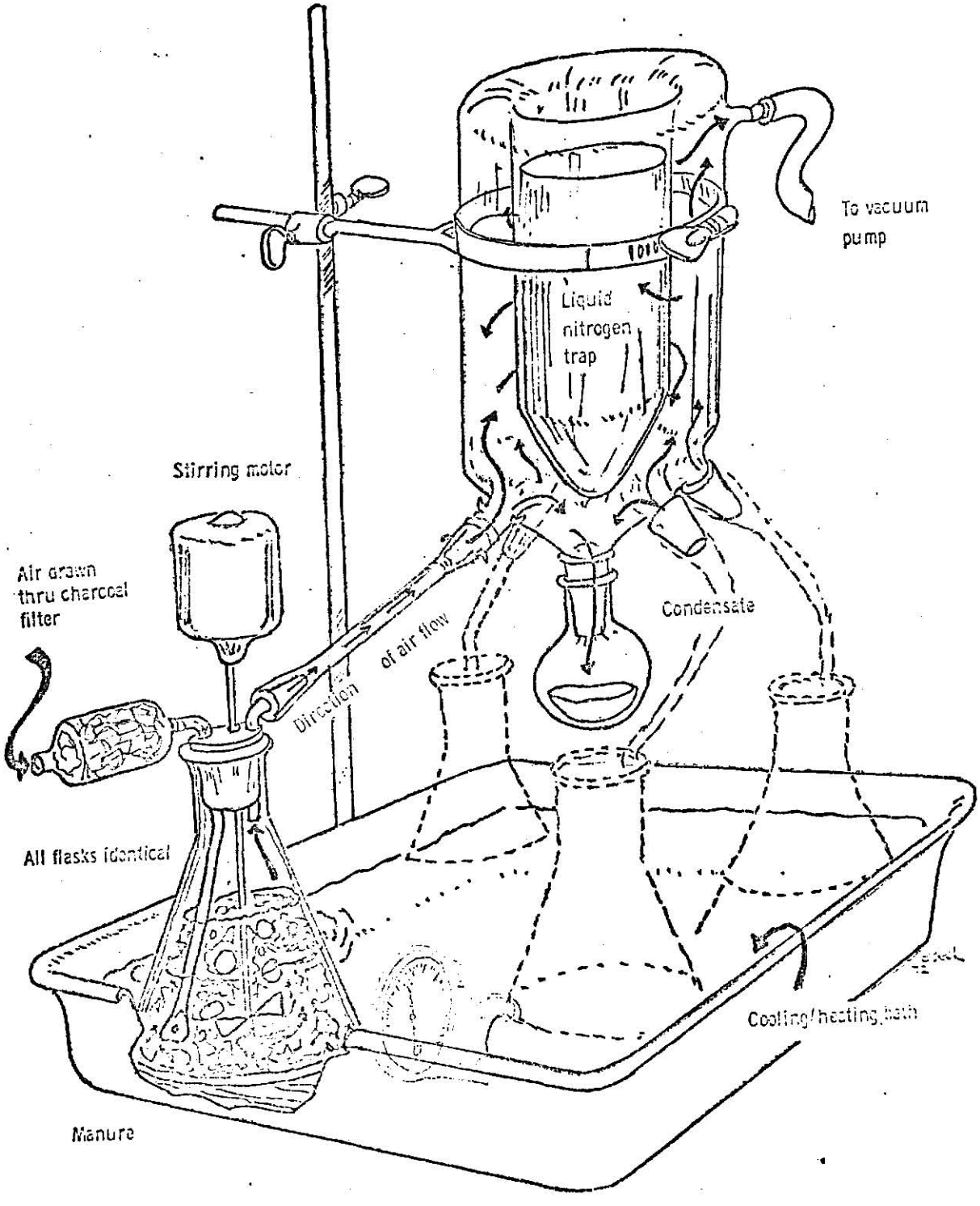
The attraction of flies to manure is thought to be accomplished through the medium of an odor. It has been observed that while flies are attracted to fresh samples of manure, after the surface of the manure has dried and a crust forms, the flies no longer are attracted. The reason for this lack of attraction may be due to the crust preventing the volatile odor-material from leaving the manure. It has been shown by various workers that there are many volatile compounds in the manure of different species of animals and fowl. The listing of the volatile compounds as reported in the literature at the present time is given on page 19. (White, 1972). Odor measurement by analytical methods has been hindered both by the low concentration of odorous compounds at their odor threshold, and by the difficulty in separating and identifying odorous components. Developments in gas chromatography have provided researchers with a method of separating and identifying odorous compounds. Positive identification of the separated fractions requires either mass spectrographic or infrared analysis. Similar gas chromatographic retention times or volumes can only be considered tentative identification.

Table one; Volatile compounds from nature as reported in the literature.

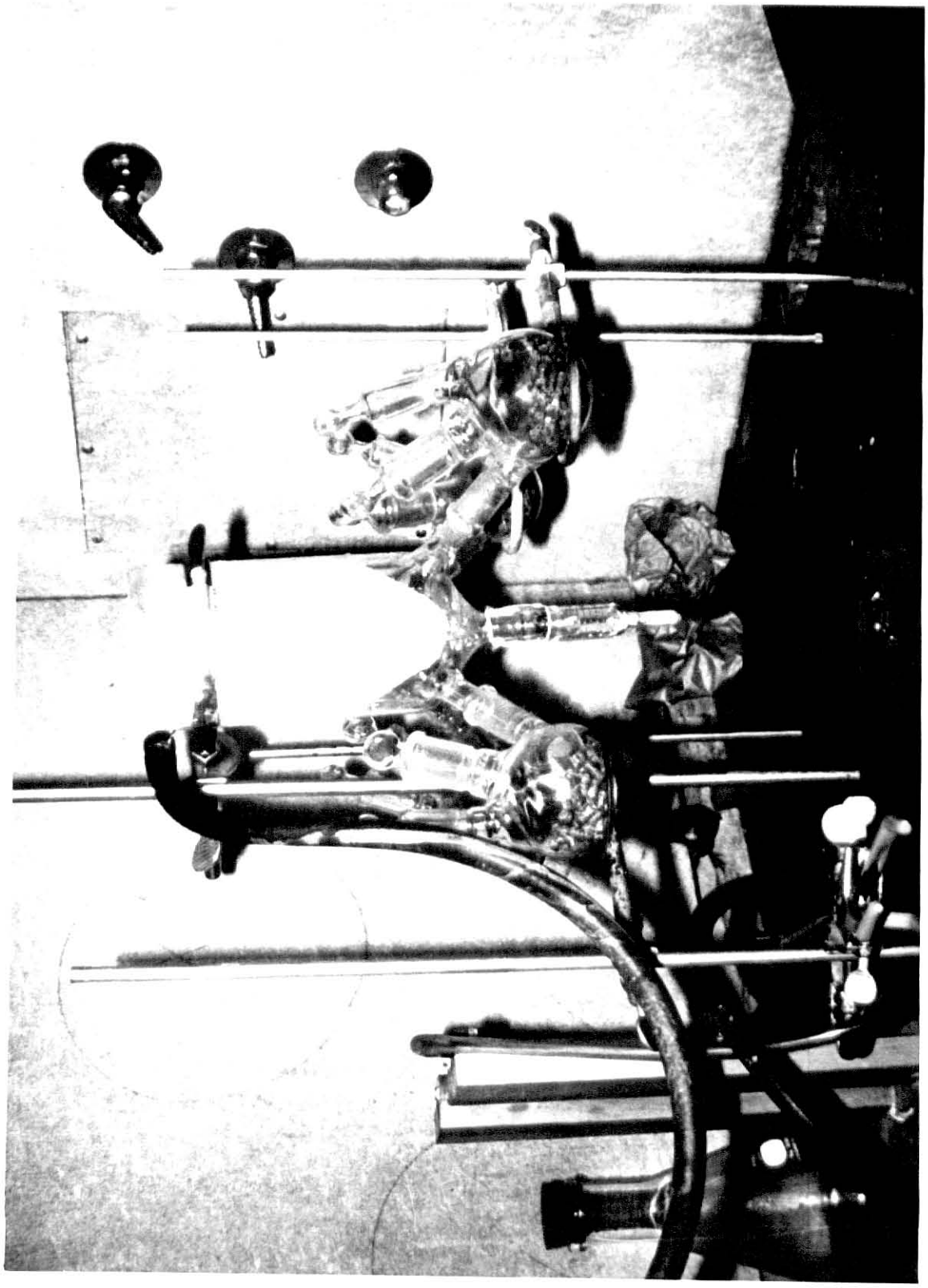
Compound	Animal
ammonia	swine, cattle
water	swine
carbon dioxide	swine
sulfur dioxide	swine
methanol	swine
ethanol	swine
1-propanol	swine
2-propanol	swine
1-butanol	swine
isobutanol	swine
isopentanol	swine
butanoic acid	chicken
3-hydroxy-2-butanone	chicken
acetic acid	chicken
propionic acid	chicken
isobutanoic acid	chicken
hydrogen sulfide	chicken, cattle
methanethiol	chicken, cattle
ethanethiol	chicken
dimethyl sulfide	chicken, cattle
n-propanethiol	chicken
2,3-butanedione	chicken
n-butanethiol	chicken
indole	chicken
3-methyl indole	chicken
dimethyl sulfide	cattle
propyl acetate	cattle
n-butyl acetate	cattle
trimethylamine	cattle
methylamine	cattle
ethylamine	cattle
dimethylamine	cattle
n-propylamine	cattle
isopropylamine	cattle
n-butylamine	cattle
n-pentylamine	cattle

Early work done on this project, by other workers, involved the search for a method of trapping the volatile materials from manure samples and the attempt to obtain reproducible results through the means of gas chromatographic separation of the components. The method for collection cited most often in the literature, was that of head space analysis. In general, this technique consists of sampling 'equilibrium' vapors in a closed container with a gas-tight syringe. Although the technique is simple and relatively rapid, it does suffer from interference due to vapor pressure predominance by the major components collected, (i.e., water and carbon dioxide). (Mendelsohn, Steinberg, and Merritt, 1966). Other problems encountered with this technique are low sample concentration of high-boiling compounds (Johnson and Nursten, 1971) and decreased peak resolution experienced when large volumes of head space gas are injected (Sully, 1971). This method, for all of its limitations, was initially the one of choice, however, because one knew of no better way to do it. A sample of cow manure was lyophilized in either of two ways. A commercial lyophilizer was used to deal with large samples of cow manure or a smaller, less efficient version, known as a COW, was used for small samples. The COW is a glass, fraction collector which has provision for a liquid nitrogen trap. A drawing of the experimental assemply of the COW is shown on page 21. The method involves the collection of volatiles

Figure two; COW assembly used in the extraction of the volatile materials from manure samples.


To vacuum pump
Liquid nitrogen trap
Stirring motor
Air drawn thru charcoal filter
Direction of air flow
Condensate
All flasks identical
Cooling/heating bath
Manure

Photograph four; COW assembly used in the extraction of volatile materials from manure samples.



by drawing air over a sample of manure and then passing the air through a condenser with dry ice and acetone as coolant. The condensate is collected and the volatiles in the head-space gas are allowed to warm to 60°C. A 1 ml sample of the head-space gas is injected into a 20% carbowax on chromasorb-P acid-washed, packed column. The gas chromatogram which results from volatiles collected by this method is shown in spectrum 1 on page 24. The chromatograms obtained in this way suffered from lack of reproducibility. This may have been the fault of the method or of the samples. Various methods of modification of the method were attempted, such as the use of an activated charcoal or a silica gel filter placed in apparatus to adsorb out the water and the high-molecular weight organic volatiles which seemed to cause some interference. This met with little success, however, and after repeated failures to achieve reproducibility, the method was abandoned in favor of the one described later in this presentation.

Some time was spent in the early stages of this project, with an attempt to identify some of the peaks on the gas chromatogram shown on page 24, from retention times obtained by the injection of the known volatiles in cow manure as listed in the table on page 19. This was found to be a very time consuming and difficult task, however, since even the very pure compounds injected resulted in more than one peak on a gas

Spectra one; gas chromatogram of the odors trapped from a sample of cow manure using the COW method of extraction. The chromatogram is in two parts, on this and the following page.

Column--Carbowax 20M on Chromasorb P, acid-washed, DMCS treated

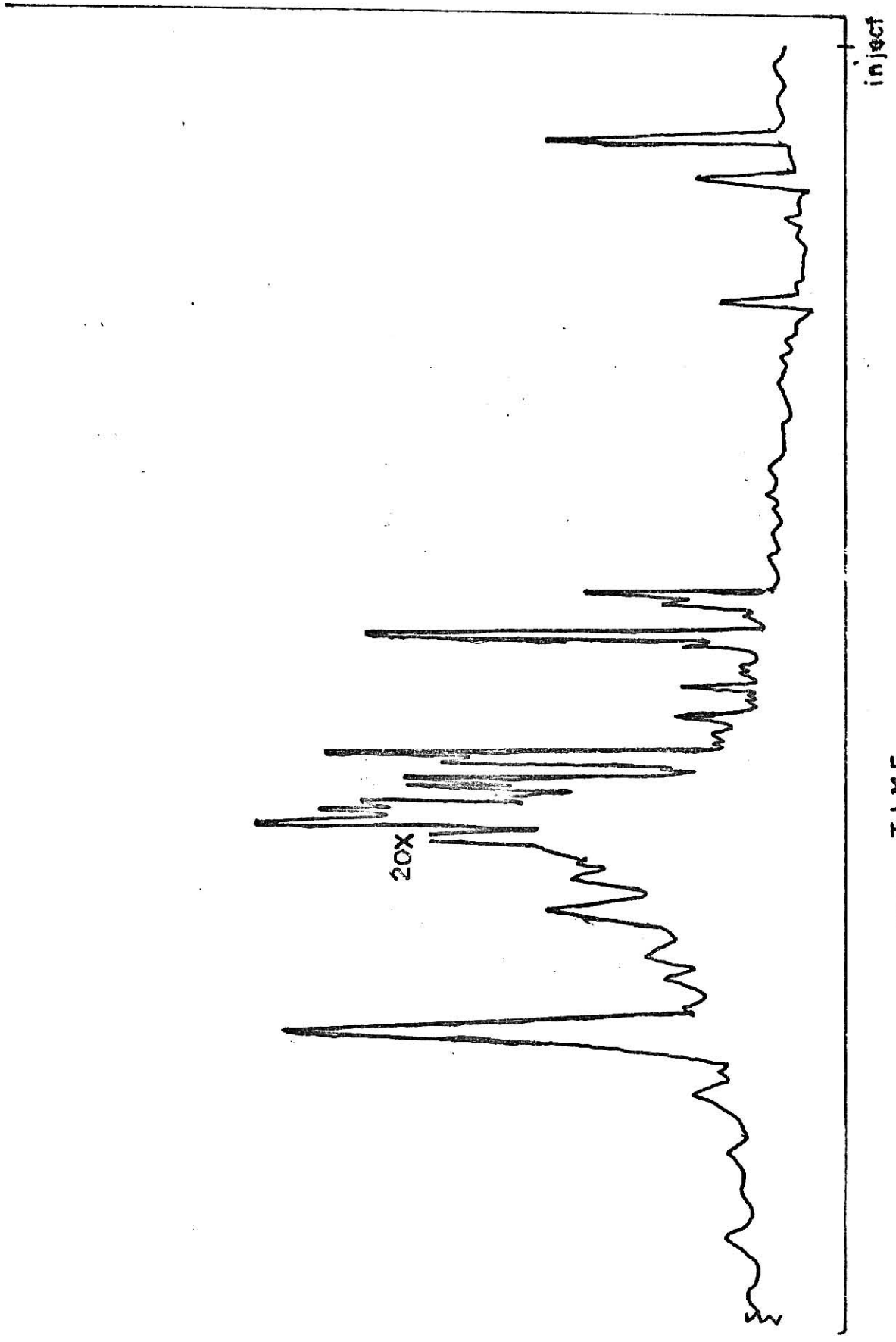
Detector--FID

Temperature--Isothermal--100°C

Column length--10 ft X 3/16 inch O.D.

Carrier gas--Nitrogen

Carrier gas flow rate--20 ml per minute.


inject
20X
TIME

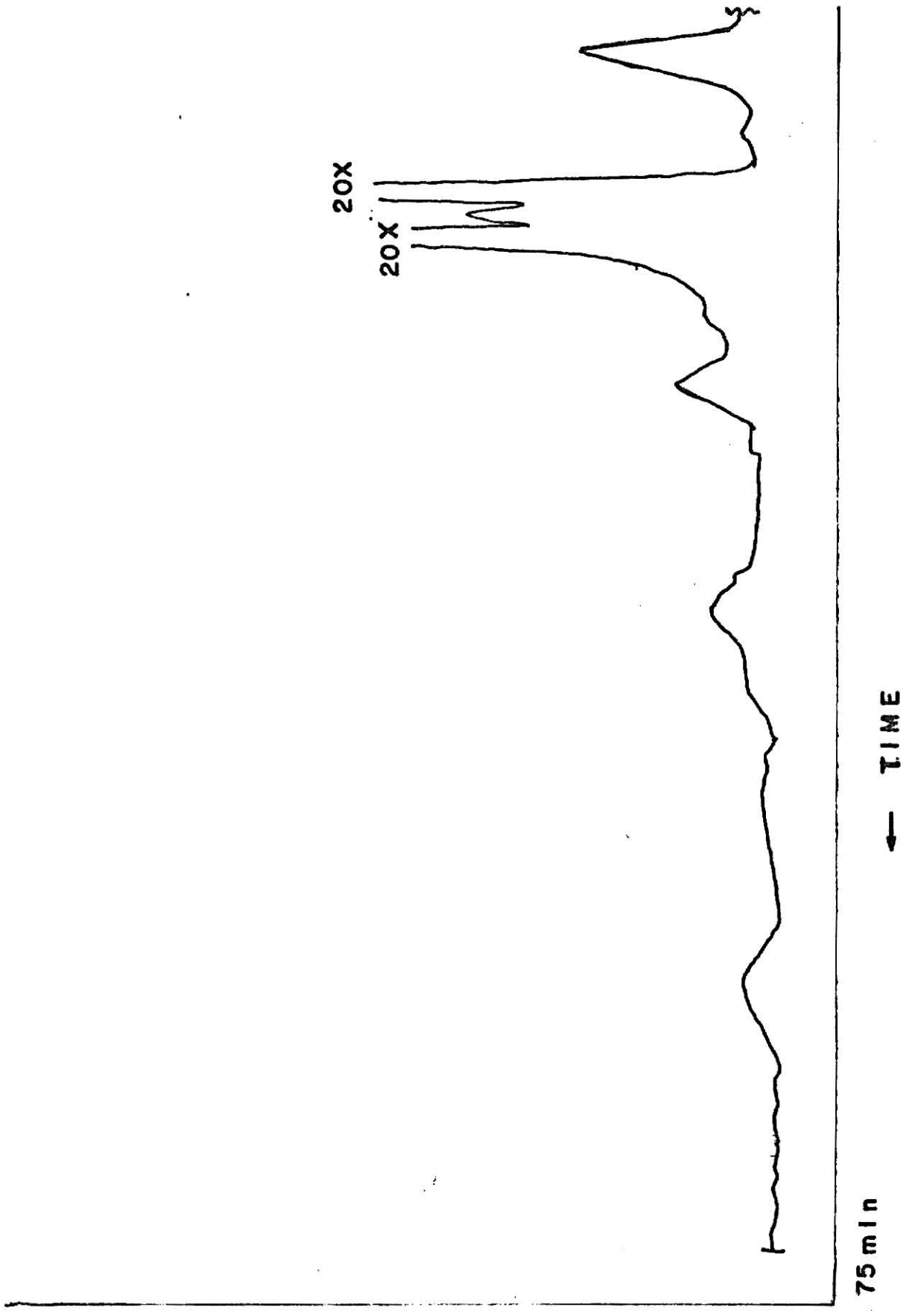

20X
20X
TIME
75 min

Figure three; the Bay Olfactometer.

A--air inlet

B--flowmeters

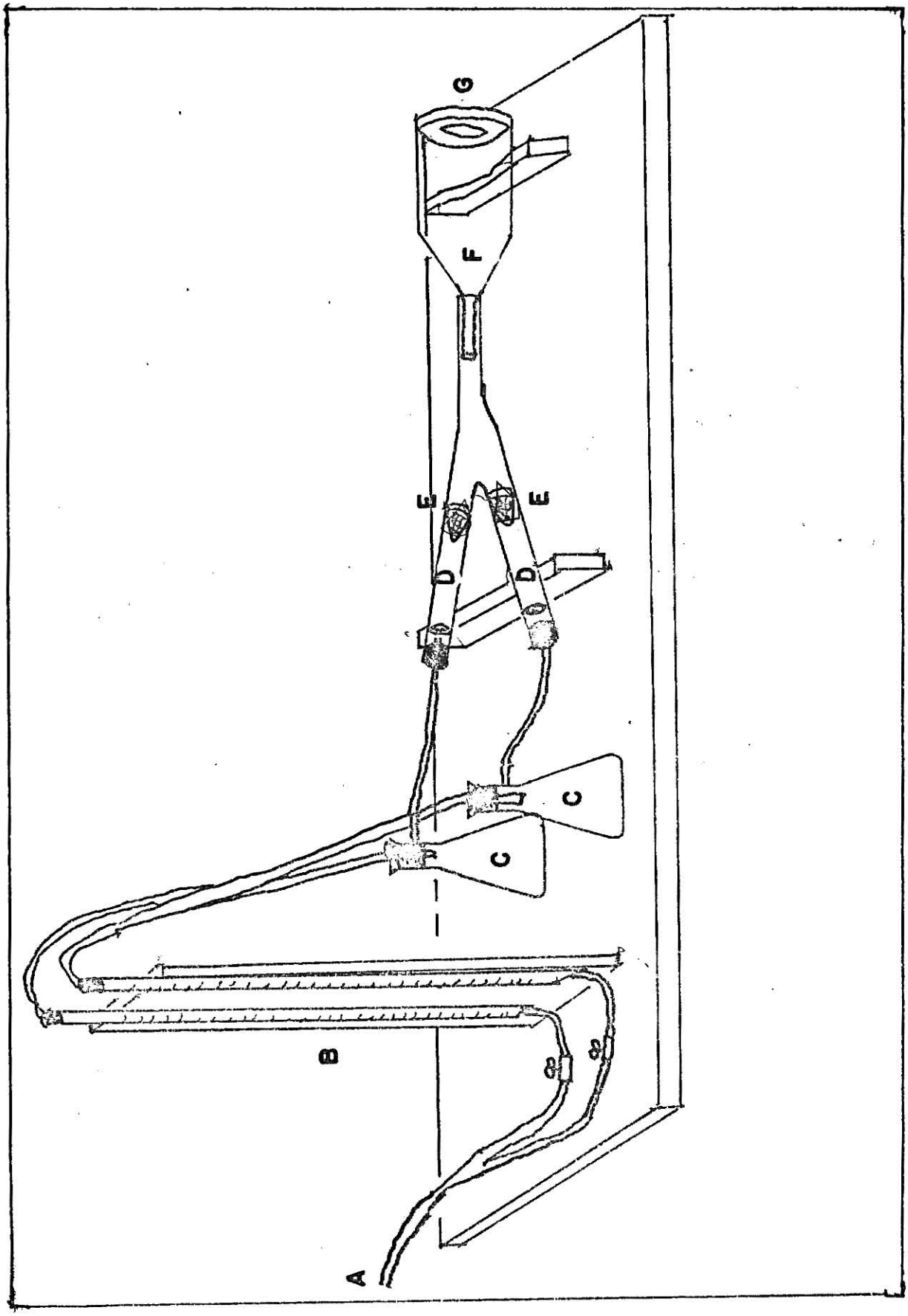
C--sidearm flasks

D--arms of trapping device

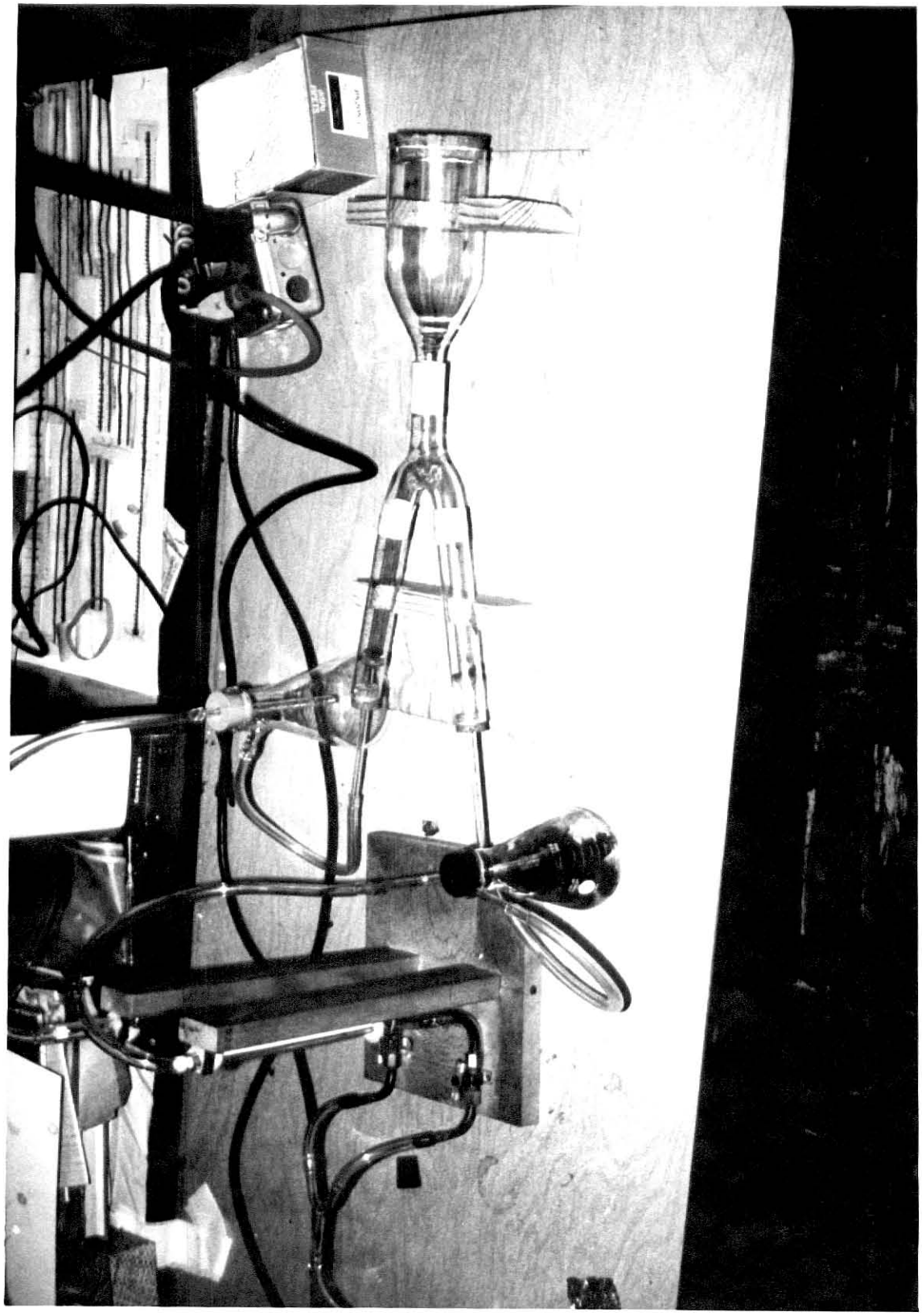
E--one-way screen funnels

F--holding chamber for flies before testing

G--large cork with screen for exit of air passing through system.


G
F
E
E
D
D
C
C
B
A

Photograph five; Bay Olfactometer



chromatogram.

It is known that cow manure is made up of nearly 83% water. This very large amount of water caused such dilution of the condensate by the lyophilizing methods used that results of fly tests with the condensates were not at all reproducible. Attention was directed to the determination of just where the attracting material was first formed in the animal, if it indeed was not present from the very food itself. The answer to this question could only come from the flies which were ready to lay their eggs, themselves. A method for the detection of fly attracting odors had been formulated by Dr. Pitts and his student, Darryl Bay, along with the apparatus which would allow for use of the method. The apparatus is shown in the diagram on page 25. The detection device is known as an ofactometer and is used as follows: A number of gravid female flies are placed in the large bulb and a sample of manure or other odor producing substance is placed in one of the side-arm flasks. The air pump is turned on and the flow rate is adjusted to 2000 ml per minute. The air is blown over the odor containing material and into one arm of the olfactometer, while air that does not contain any odor is blown through the other side-arm flask and into the other arm of the olfactometer. This serves as a reference and control, since both arms of the device do not have air passing through

them. The arm tubes of the device have screen funnels placed into them, in a manner which allows flies which are attracted into the arm tubes to enter the tube, but makes it difficult for them to leave again. This allows them to be counted only once and yields more accurate results. It was found that this device provided a very easy and quick method of determining whether or not a sample placed in the side-arm flask did indeed contain an odor which was fly-attracting and was used throughout this project as a detection device and method. It was found, through its use, that a manure sample collected from a bull on a predominantly hay diet would attract in excess of 80% of the total flies placed in the large bulb in less than five minutes, consistantly. Varying numbers of flies were used in any given trial, the number always being recorded, along with the number found in each side of the olfactometer and the number of flies which did not react at all from each trial. These numbers will be found accompanying each of the trials given later in this presentation.

Ten samples from physiologically different places in the intestines and stomach of two bulls butchered by the meat department of Kansas State University were collected by Dr. C. E. Meloan and one of his students, M. Anne Liedtke, in order to attempt to answer the question of the origin of the fly attracting odor in the manure. The samples were tested on the

flies with the olfactometer. The results of these tests were the indication of the presence of the attractant material being found in the first portion of the rumen and at each of the stages being sampled after that. Ten samples were collected from each of the two bulls, the location of sample collection being listed on the following page. The results were the same for both bulls with respect to the fly tests.

Gas chromatograms were obtained on a few of the fractions, using the COW method previously described. The chromatograms showed little difference in the number or location of the peaks. The only difference appeared to be in some of the peak areas. This study offered little in the way of identification, but did give evidence that the fly attracting material is present either in the food itself or is formed in the very first portion of the digestive tract, since fly attraction tests on the contents gave positive results for samples taken from the rumen and each sample tested thereafter. There appears to be some possibility that the attracting material may be a metabolite formed during the initial digestive process.

A method of utilizing the undigested stomach contents of butchered animals, as feed for cattle, has been tried by some feedlot managers, experimentally. In this method, the undig-

Samples From Digestive Tract Taken

From Two Different Bulls

1. Rumen
2. Reticulum
3. Omasum
4. Abomasum
5. Duodenum
6. Combined small intestine
7. Secum
8. Spiral colon
9. Descending colon
10. End of colon

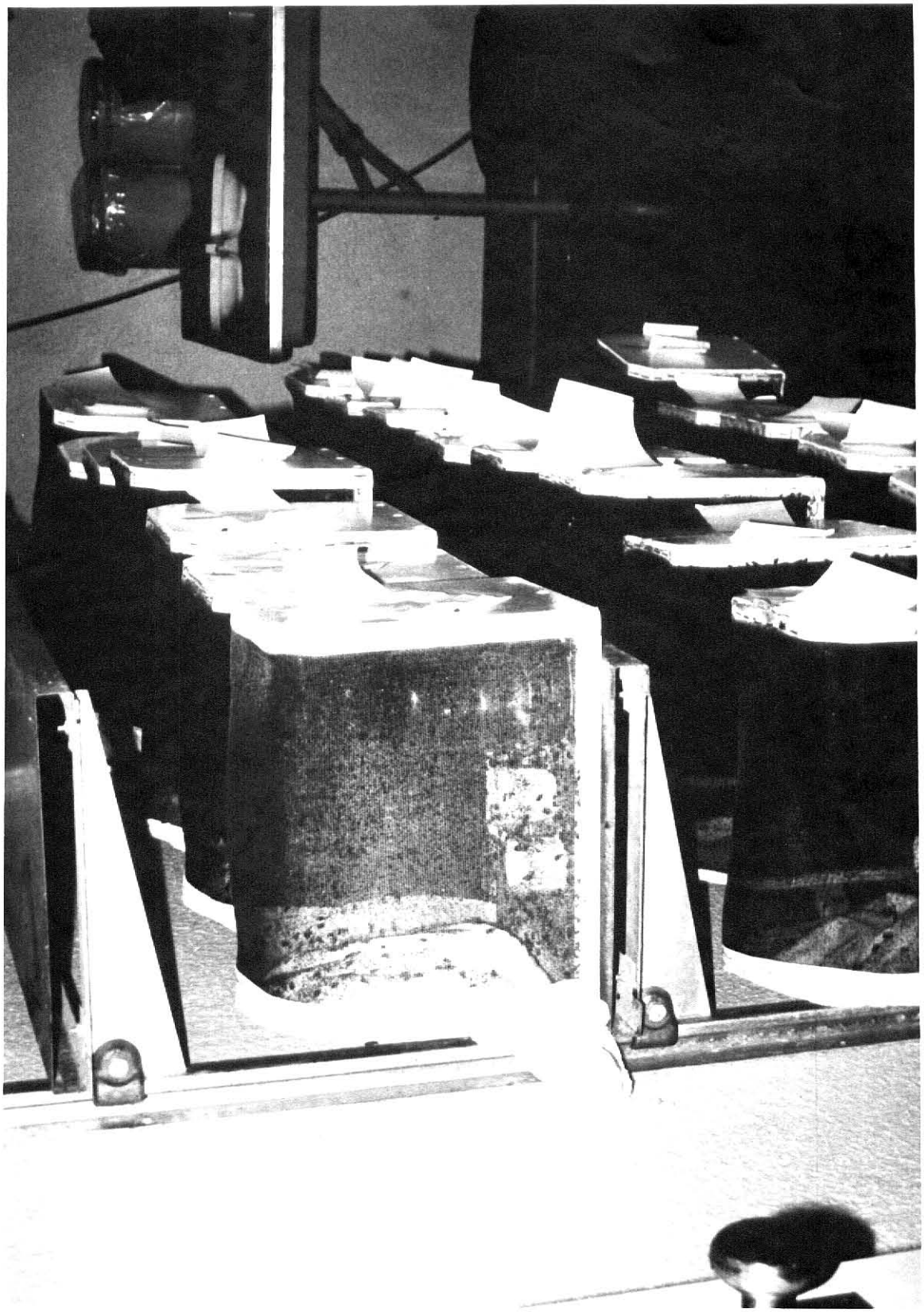
Fly tests were positive for all samples, although no tests were more indicative of attractive material than others. The only positive result is that the attractive material is present from the rumen throughout the digestive tract.

ested contents of the stomach are steamed, to kill the bacteria, then compressed into pellets and fed to cattle as a diet supplement. A sample of these pellets, termed 'paunch pellets', commercially was obtained and when tested on the female flies using the olfactometer, was found to contain the attractant to a large degree. The response to the odor from the pellets was on an order approaching that of the fresh manure samples tested previously. Here again was evidence that, although bacterial action may enhance the production of the odor which attracts the flies to the manure, the odor producing material may be present before ingestion, since steam-killing of the bacteria from the stomach did not destroy the attractant odor.

The photograph on page 33 shows the fly rearing room in the Entomology Department at Kansas State University. The flies were provided under the auspices of Dr. Charles W. Pitts. The fly room is maintained under very close tolerances as to humidity, temperature and lighting. These factors all influence the egg-laying and life cycle of the flies. The flies used in this project were face and house flies. These flies are easily raised, are prolific and have a nearly consistant time span from hatching to egg-laying period. They are known to be present in cattle-breeding and feedlot areas, and in urban communities. The face flies feed under and a-

round the eyes, and in and around the nostrils, and at the lips of animals. They suck blood and other exudates from the surfaces of animals, but cannot pierce the skin. (Scott and Littig, 1960).

Photograph six; Fly rearing room, Entomology Department, Kansas State University.



Photograph seven; A cage of face flies, as used in the tests conducted in this problem.


ENT

Part II

The early work done on this project had accomplished little toward the isolation and identification of the compound or compounds responsible for the attraction of flies to manure. It was decided to redesign the apparatus. Two things were thought to be needed. These were, (1) to continuously cycle the vapors and (2) to chemically trap them. The cycling apparatus was built and a place for the chemical adsorbant provided. The problem was--what to use as an adsorbant? During a discussion of the project with a member of the Entomology Department one day, mention was made of a new porous polymer packing material for gas chromatographic columns which was reputed to be very good for the adsorption of volatile organic compounds. It was decided that a sample of this material would be obtained and a test of its suitability made. The new packing material is sold under the trade name of Tenax-GC.

The trapping system used in the initial stages of this work is shown on page 37. Columns for the gas chromatograph were fabricated of glass and packed with Tenax-GC. In the remainder of this presentation, Tenax-GC will be referred to as simply, Tenax. The initial columns used were 3/16" O.D., by

30 inches long. After trapping the odors from a manure sample, which was stirred manually from time to time, for a period of twenty-four hours, the sample was injected into the gas chromatograph by means of the system shown on page 39 The special items required for use of this system are also shown on the following pages. A gas chromatogram was obtained which had fewer peaks than those obtained by the system which had been used previously. This was heartening, in that it was thought that it may be less difficult to isolate the peak or peaks responsible for the attraction of the flies to manure by use of this simpler chromatogram.

The sample is injected into the gas chromatographic column by using the injection apparatus as described below.

The Tenax containing the trapped volatiles is placed in the glass cylinder and the glass cylinder inserted into the brass injection tube. The heater is a device formed by first wrapping the brass cylinder with two layers of asbestos paper, allowing it to dry, wrapping with a number of turns of 20 B&S gauge chromel wire, then further wrapping with a sufficient number of layers of asbestos paper to prevent the outer surface from becoming hot enough to char. This required, in those heaters constructed for this project, approximately ten layers of asbestos paper for the outer covering. The brass

Figure four; Trapping system used in the early stages of the development of a method using Tenax-GC as a trapping medium.

A--side-arm flask

B--heating mantle

C--stirring motor

D--Tenax-GC trap

E--Centrifugal pump, mechanically driven

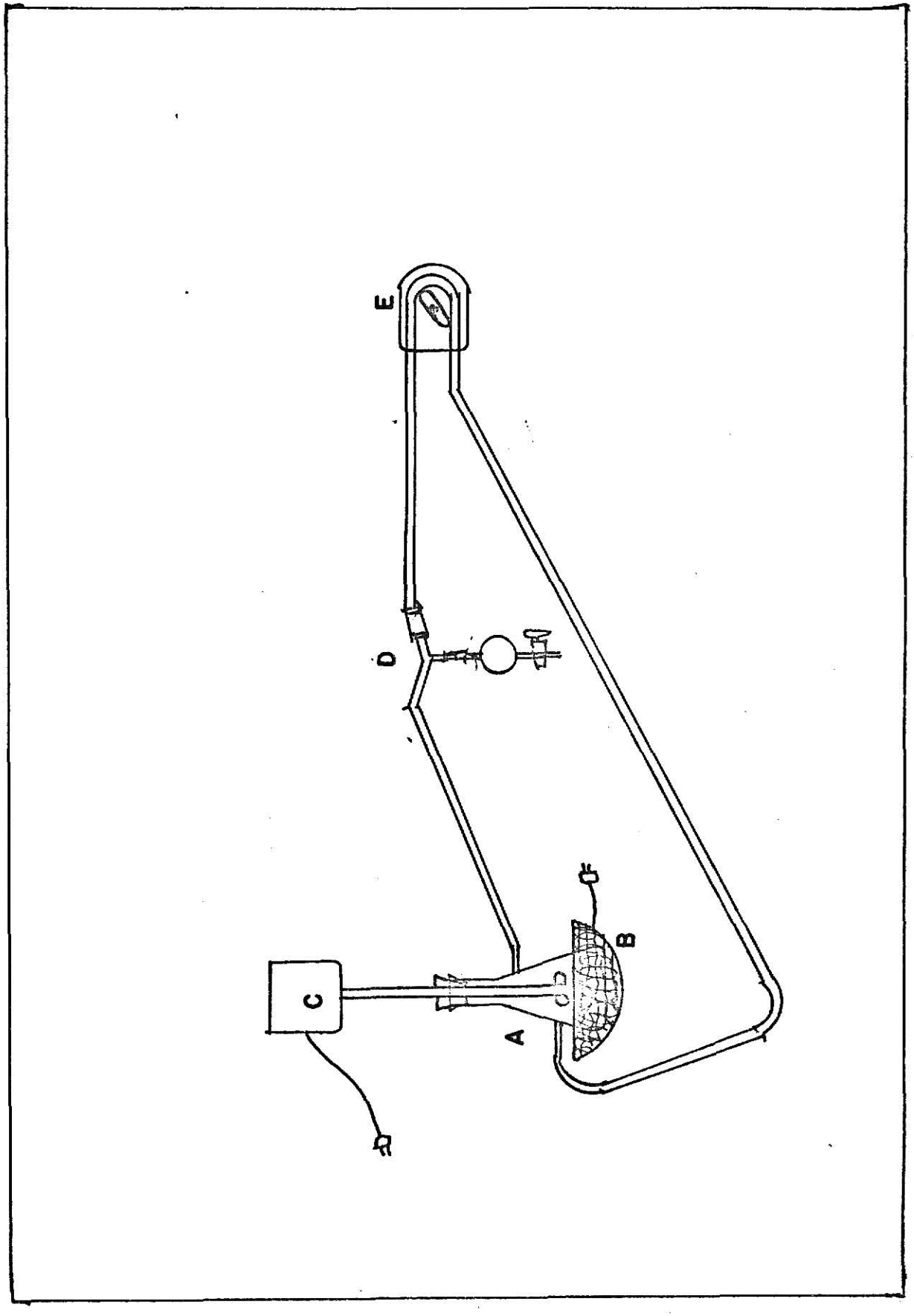

E
D
B
C
A

Figure five; The Tenax-GC trap used initially.

A--bulb for collection of water from sample

B--two-way stopcock, glass, for removal of trapped water

C--fritted glass disks

D--glass ears, for securing collector parts

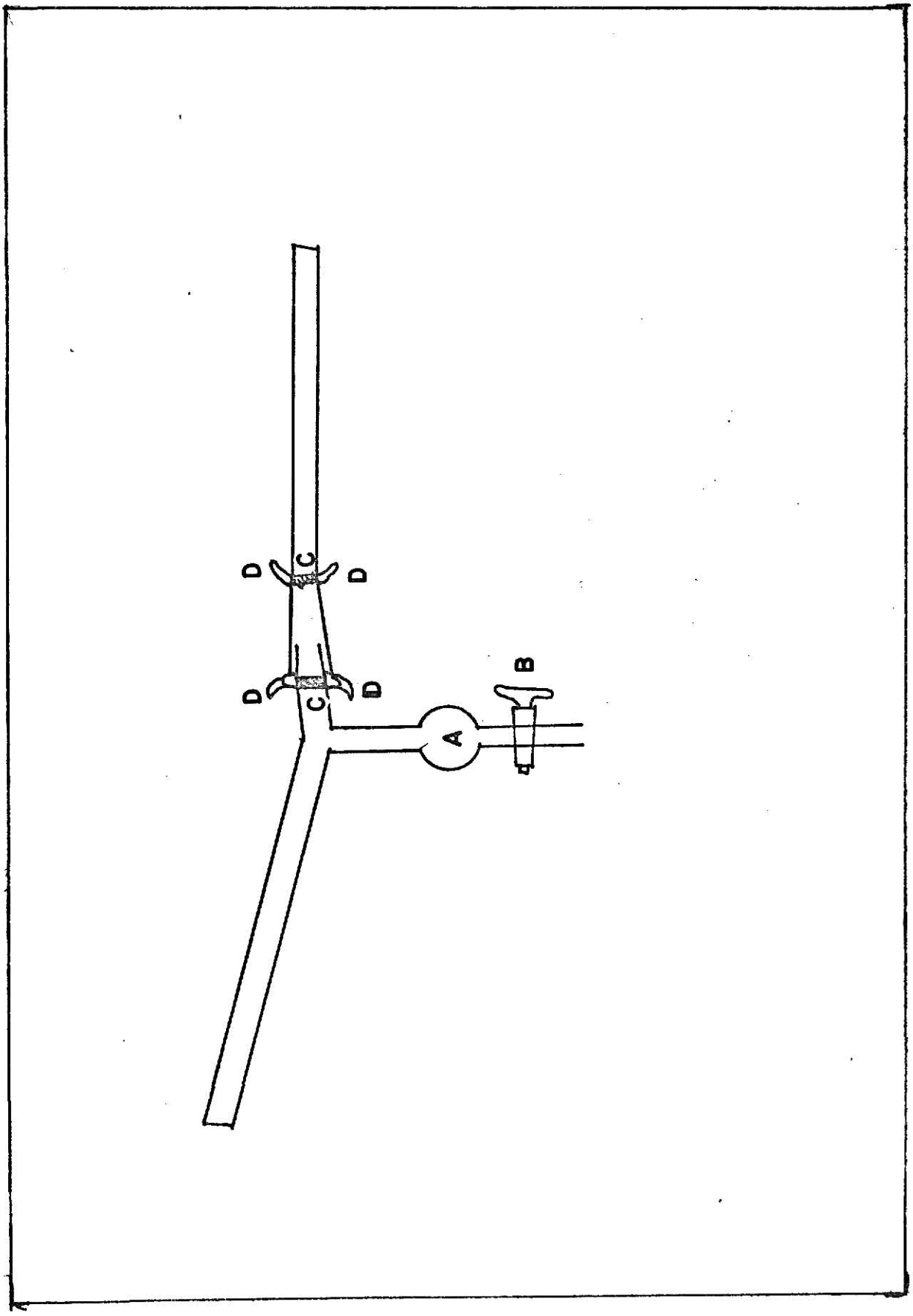

D
C
D
D
C
D
B
A

Figure six; device used for introduction of the sample into the gas chromatograph.

A--fitting for connection of the nitrogen purge gas line

B--cap used when introducing sample into gas chromatograph

C--glass cylinder containing Tenax-GC

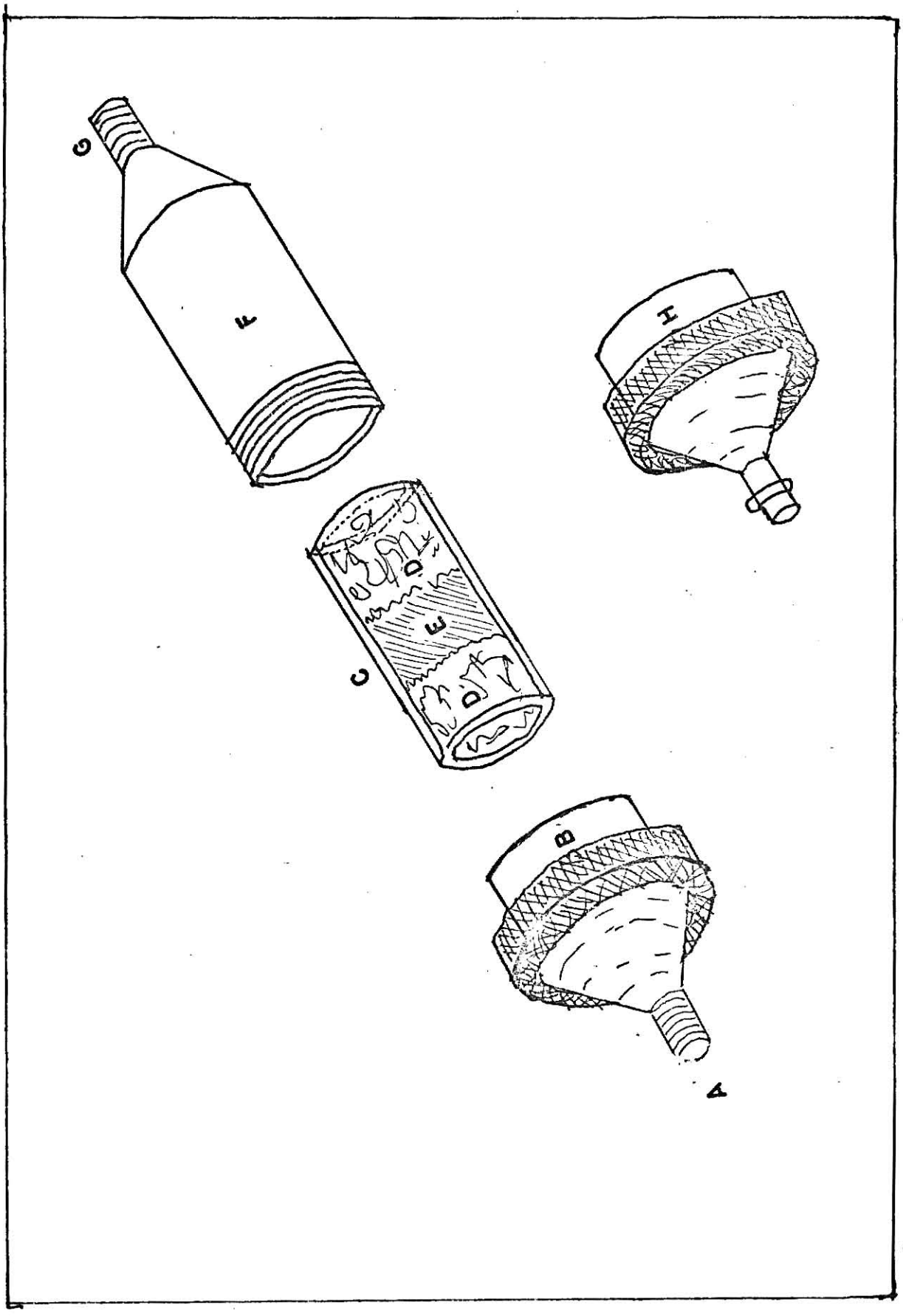
D--glass wool used to hold Tenax-GC in position

E--Tenax-GC

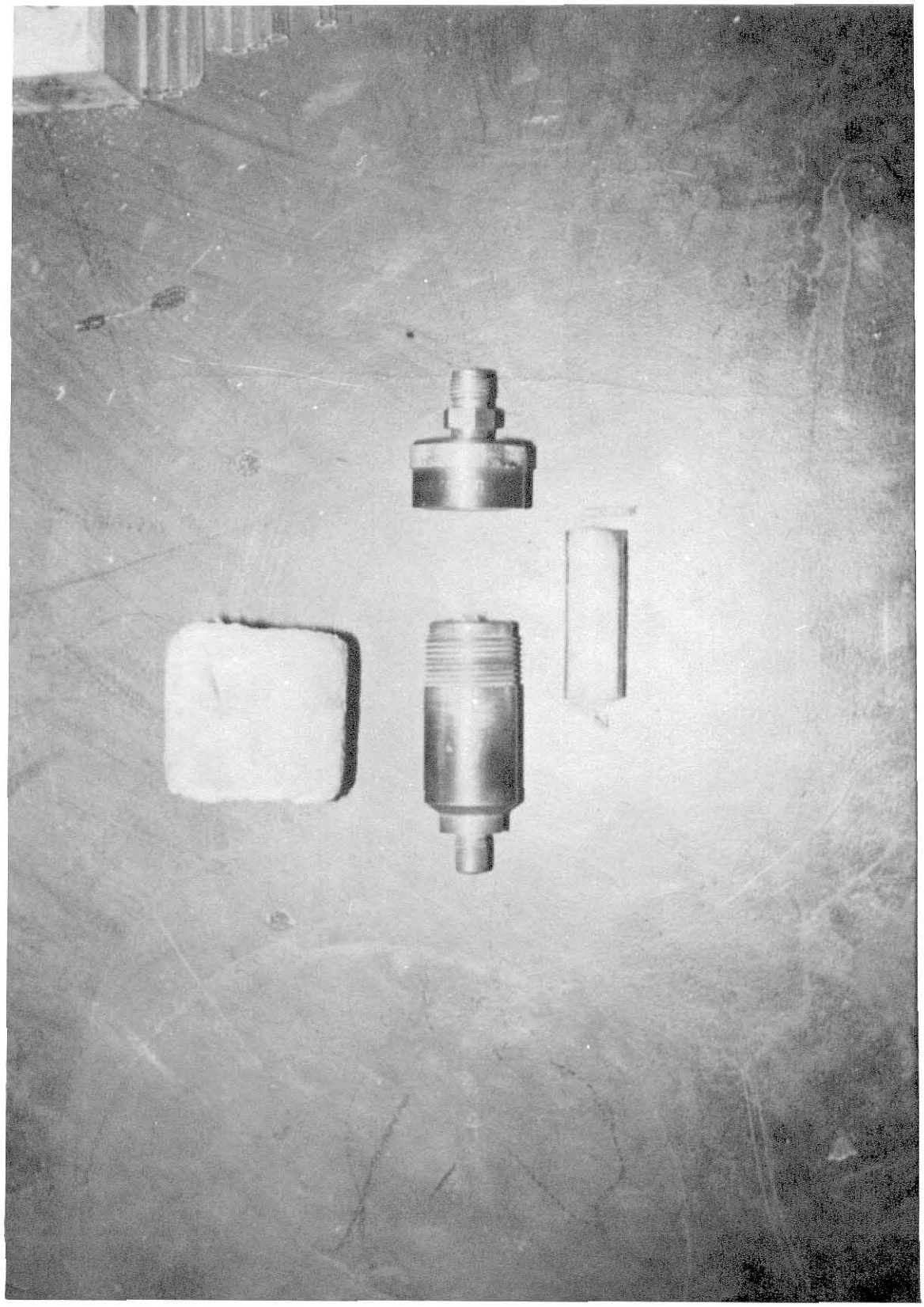
F--body of brass cylinder into which the glass cylinder is placed.

G--fitting for connection of injector to injection port of the gas chromatograph

H--cap used when collecting the volatiles on the Tenax-GC


G
F
I
C
D
E
B
A

Photograph eight--injection device, showing the brass cylinder, the cap used during injection into the gas chromatograph, the glass cylinder containing the Tenax-GC, and the heater used to raise the temperature sufficiently to release the trapped components from the Tenax-GC.



cylinder-heater assembly is screwed into the injection port of the gas chromatographic oven and the trap inside the gas chromatographic oven surrounded by a dry ice cooling bath. The dry ice is contained in a styrofoam dish made to fit the trap. The heater is connected to a variac and the probe of a thermocouple temperature gauge inserted between the heater jacket and the surface of the brass cylinder. The variac is turned on and the thermocouple used to monitor the temperature of the Tenax containing the sample, as it is being heated. During the heating process, nitrogen carrier gas is passed through the brass cylinder assembly by means of the fitting provided at the end of the cylinder. The nitrogen carrier gas flows through the cylinder at a rate of 15 ml/min. When the temperature of the brass cylinder has reached 250°C the variac is turned off and the cylinder-heater assembly allowed to cool to 100°C. This has been found to be necessary in order to allow the brass to shrink before removing it from the injection port of the gas chromatograph. It has been found that the threads on the cylinder of the injection port may be stripped in removal of the cylinder, if this is not done. The injection port septum and septum holder are then replaced and the dry ice bath removed from the trap. The trap is allowed to warm to room temperature, evidenced by the moisture condensation on the trap disappearing, and then the oven cover is closed and the sample programmed out of the trap

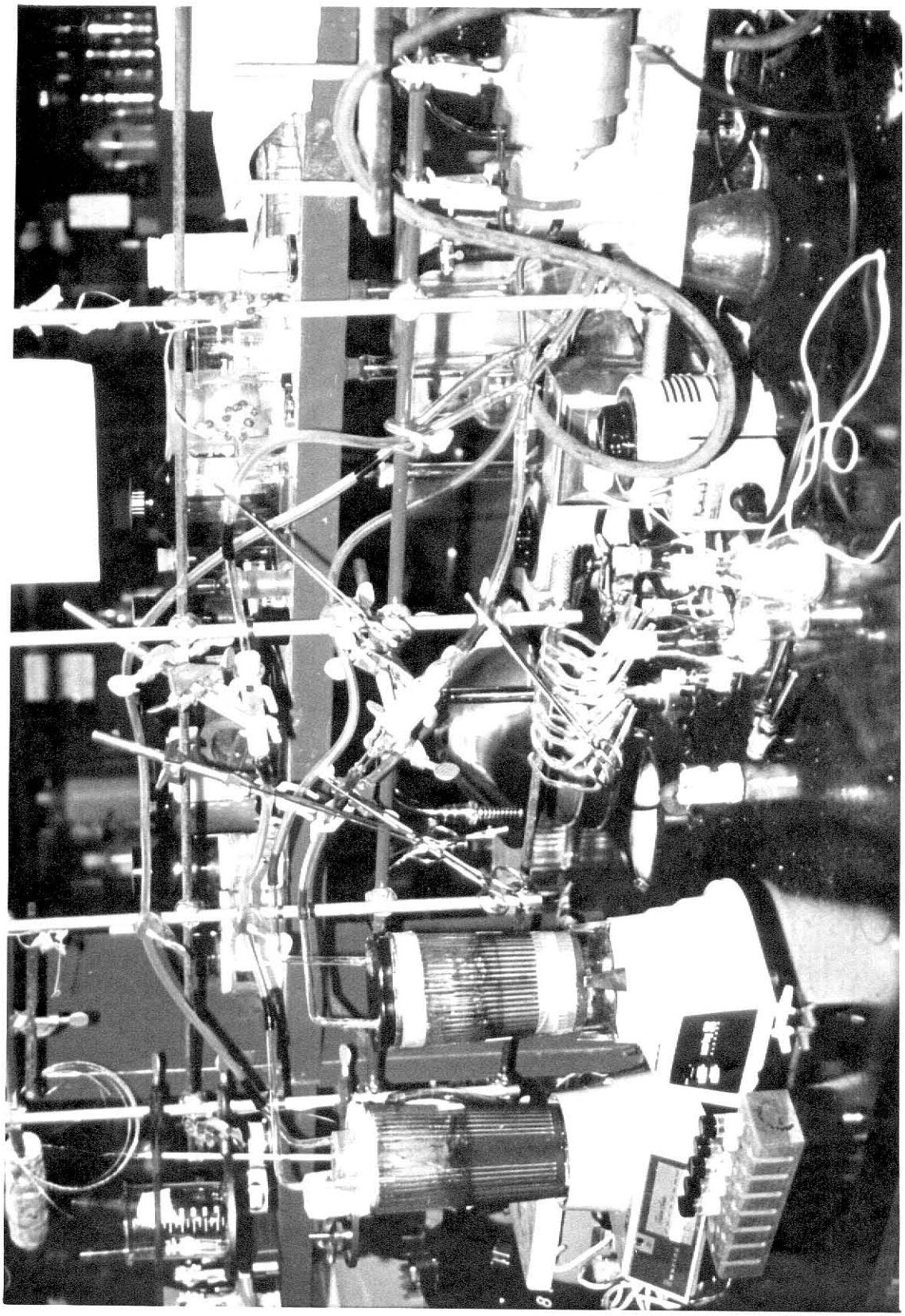
and through the column at a rate of 6°C per minute, with a carrier gas flow of 10 ml per minute. The initial experiments were performed by heating the brass cylinder and Tenax to a temperature of 350°C and programming the oven temperature to 305°C. It was later found that such high temperatures were not necessary, and the experiments were modified to the new conditions. These new conditions consisted of heating the cylinder-Tenax assembly to 250°C and the oven temperature being programmed to 250°C. The altered conditions have the advantage of prolonged column life and less baseline deviation due to temperature increase at the high-temperature portion of the chromatogram. The trap inside the oven, the connecting glass tubing to the 4-way stopcock and the chromatographic column itself, were all packed with Tenax and conditioned alike.

In order to determine whether the attracting material was present in the trapped fraction, a test was set up as follows. A portion of the Tenax containing the trapped volatiles was placed in a cage of female flies which were ready for the egg-laying process. The Tenax was placed in a small glass container, of pyrex, and the glass container put on a hotplate. This assembly was heated to 150°C. It was observed that the flies were significantly attracted to the container of packing material and that some of them actually layed

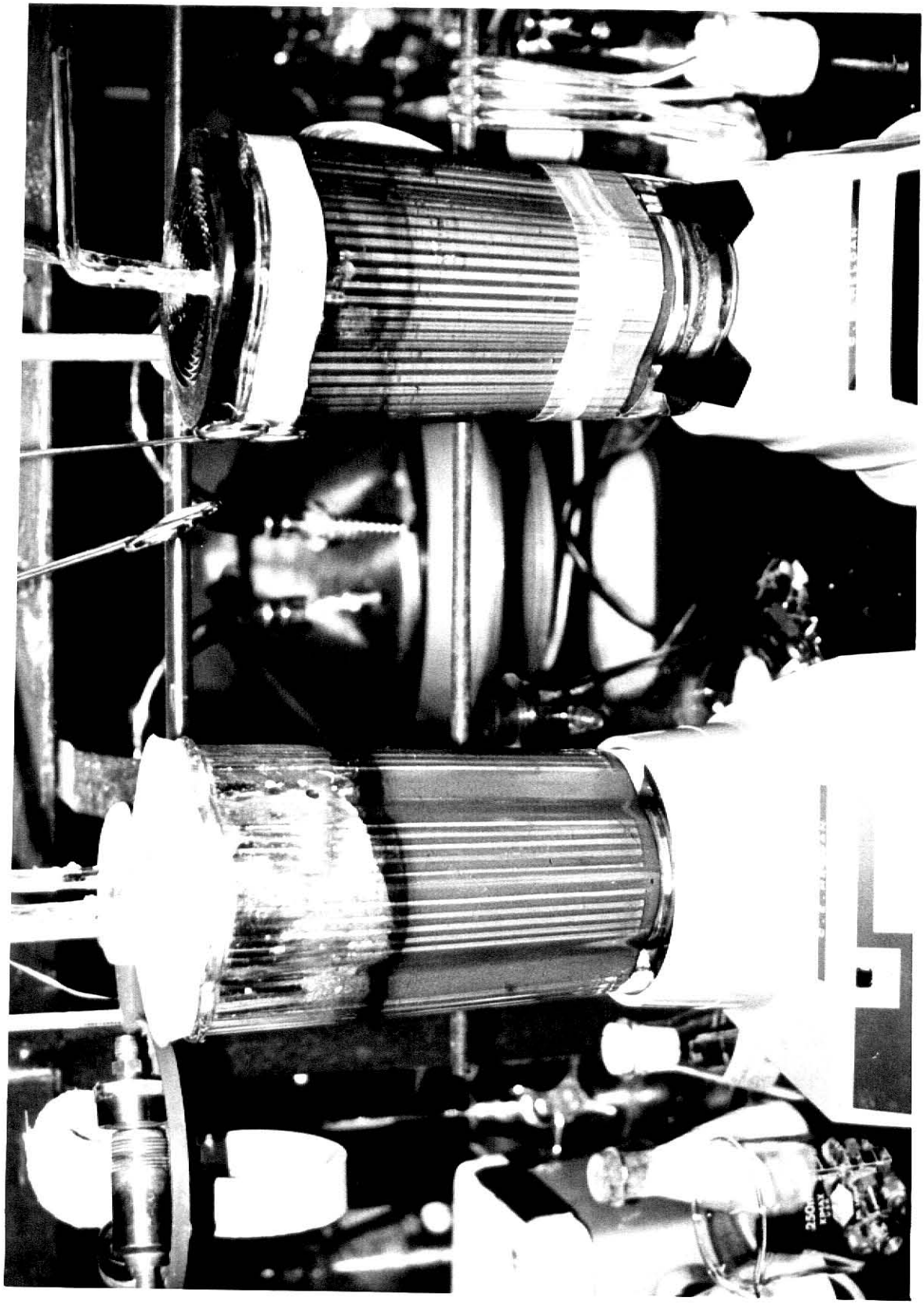
their eggs in the material. The sample of Tenax was then taken back to the laboratory and stored for a time. It was observed that the fly eggs developed into fly larvae. This was taken as evidence that the trapped volatiles did indeed contain the attracting substance and that it was present in a large enough quantity that it could be detected by the flies and that they would respond to it in the same manner that they do to a manure sample. These tests were repeated a number of times and the results were always the same. No attempt was made to count the flies attracted to the sample, since no separation or identification had been performed on the volatiles as yet. A protective wire screen was placed over the container of Tenax in tests following the first one, to prevent the flies from getting into the packing material, since it was observed that they scattered it badly and the material was lost in this manner. The Tenax used to trap the volatiles from a manure sample could be used repeatedly, if not contaminated, since the heating effectively removed the trapped components. This was determined by testing a portion of a sample which had been allowed to cool with the flies. It was found that there was no significant attraction of the flies to this sample of Tenax, only random landing of the flies on the dish being observed. The conclusion was drawn that the attractant substance could be trapped consistantly by use of this method.

The system then passed through many revisions, both in the trapping portion and the geometry of the columns and related materials used in the gas chromatograph. The final configuration of the apparatus used in trapping the volatile components from the manure samples is shown on page 48, and the apparatus used for the introduction of the sample into the gas chromatographic oven, along with the related materials used in separation and subsequent trapping of column fractions is shown on pages following. These systems offer the advantages of ease of operation, inexpensive materials and complete reproducibility. All glass-to-glass connections were made with Cajon fittings, obtainable from a number of suppliers, the ones used in this project being purchased from Kaw Valve and Fitting Co., 528 S.W. Blvd, Kansas City, Kansas. The four-way glass stopcock was purchased from Ace Glass Inc., P.O.Box 996, 639-41S Hancock St., Louisville, Kentucky, and modified by Mr. M. Ohno, KSU. It is most desirable to have any surface exposed to the volatile attractant components from the manure samples to be constructed of glass. It has been observed that if these components are allowed to come into contact with teflon, they are adsorbed and lost. Viton O-rings were found to be unable to withstand the temperatures used in this work and so graphite ferrules were purchased from Applied Science Laboratories, Inc., State College, Penn.

Photograph nine; The apparatus used to trap the volatile components from a manure sample on the Tenax-GC.



Photograph ten; enlarged view of blenders containing samples of cow manure, showing method of attachment to system.



A method now having been formulated which would allow for the trapping of the attractant material from the manure sample and the subsequent separation into the various fractions by the gas chromatographic column, the obvious next step was to narrow the chromatogram down to the region which contains the attractant peak or peaks. This was done in the following manner.

The odors from a fresh manure sample were collected on Tenax using the apparatus previously described. The volatiles were then transferred to the chromatographic column and the chromatogram taken via temperature programming at a rate of 6°C per minute with a carrier gas flow rate of 10 ml of nitrogen per minute. A typical chromatogram is shown on page 53. The chromatogram was then divided into two fractions, essentially equally insofar as the number of peaks was concerned. This gave a fraction from 25°C to 210°C and a second one from 210°C to 320°C. A second portion of the trapping material containing the odors was then chromatographed, this time instead of allowing the fractions to pass through the column to the detector, they were diverted, via the three-way stopcock at the end of the column, to an external trap composed of fresh Tenax, packed in a glass U-tube, cooled in a liquid nitrogen bath. The two fractions identified above were trapped separately and tested on female face flies using the

Figure seven; diagram of volatile component trapping system.

A--blenders

B--traps containing Tenax-GC

C--air pump, recycles air in system, no external air added.

D--variac, 110 volt, used to control pumping rate

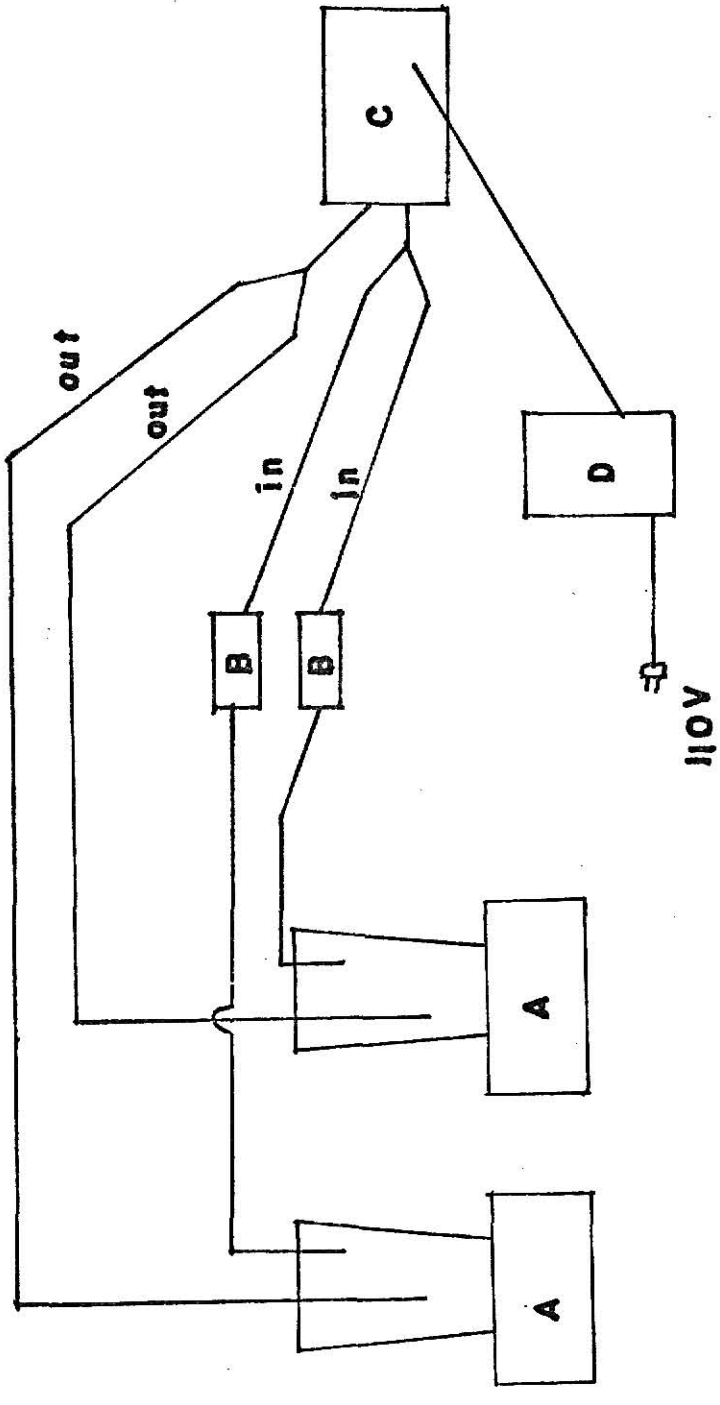

C
out
out
in
in
D
B
B
110V
A
A

Figure eight; Assembly of apparatus inside the gas chromatographic oven.

A--brass cylinder injector

B--glass U-tube trap containing Tenax-GC

C--4-way glass stopcock

D--vent

E--connection of column to nitrogen carrier gas during trapping

F--glass column, packed with Tenax-GC, 2 ft X 3/16in. O.D.

G--3-way glass stopcock

H--tubing for connection to trap from column

I--valve for introduction of nitrogen purge gas to injector

J--nitrogen gas source for purging and for carrier gas

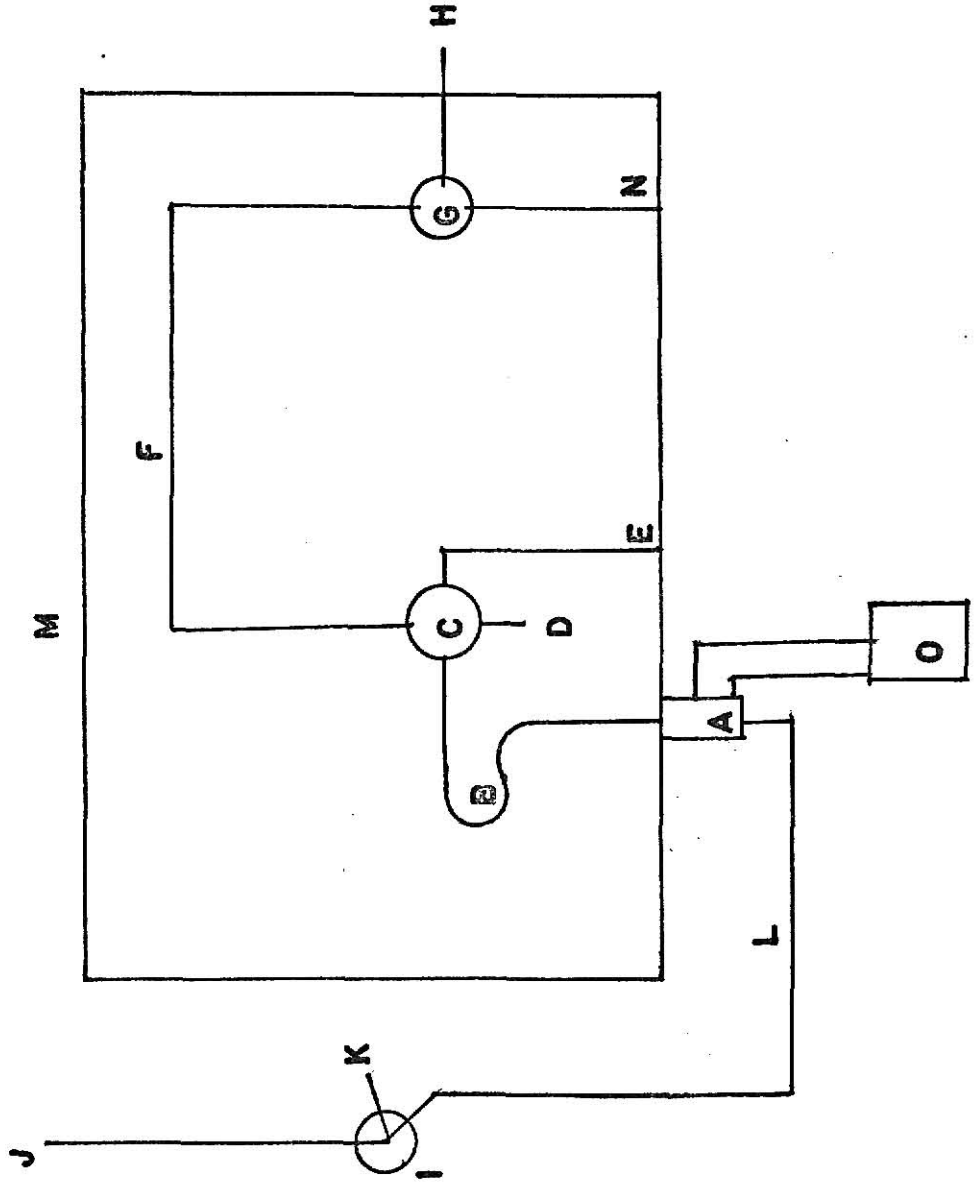
K--vent for purging gas

L--line carrying purge gas

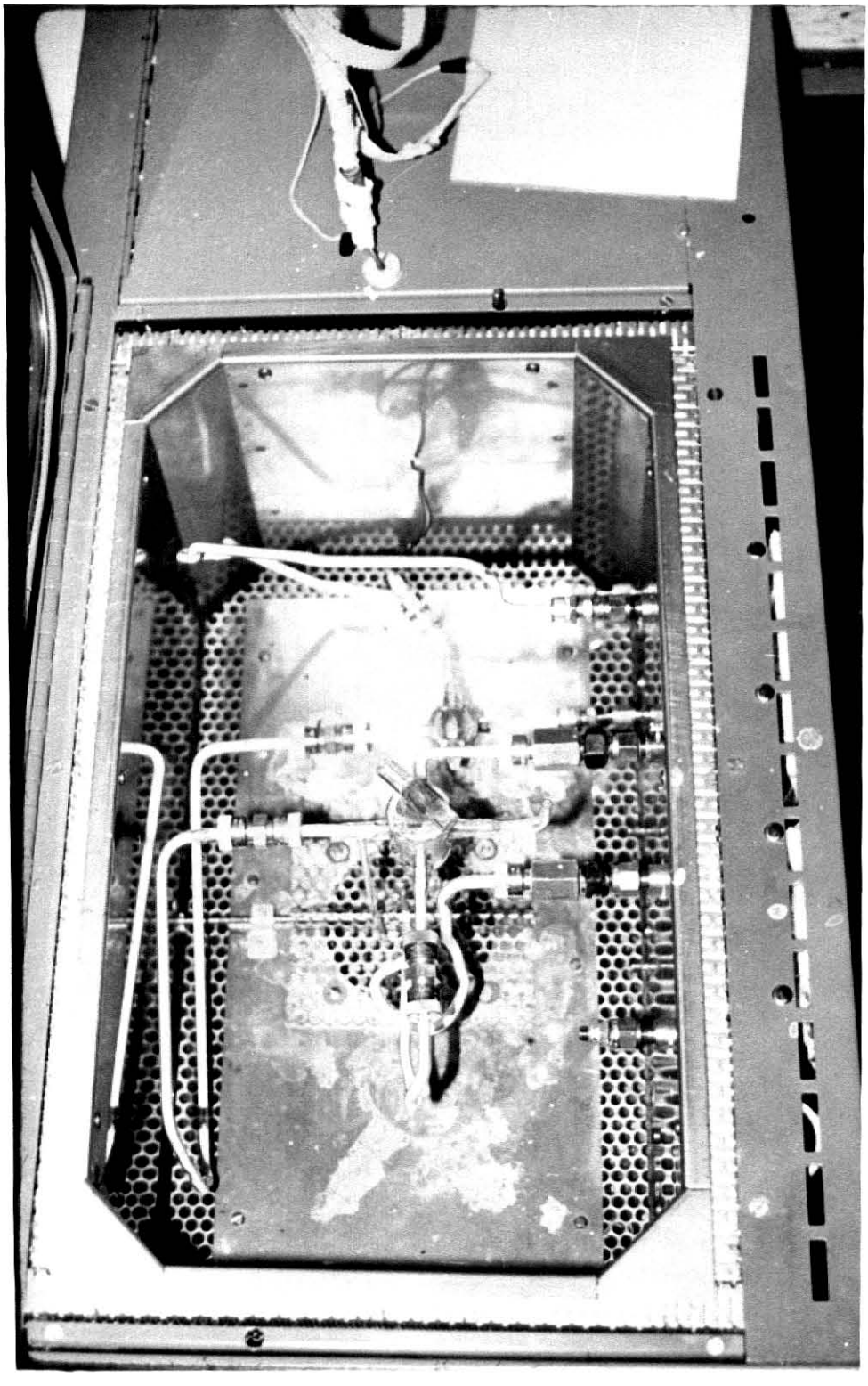
M--oven wall

N--detector port

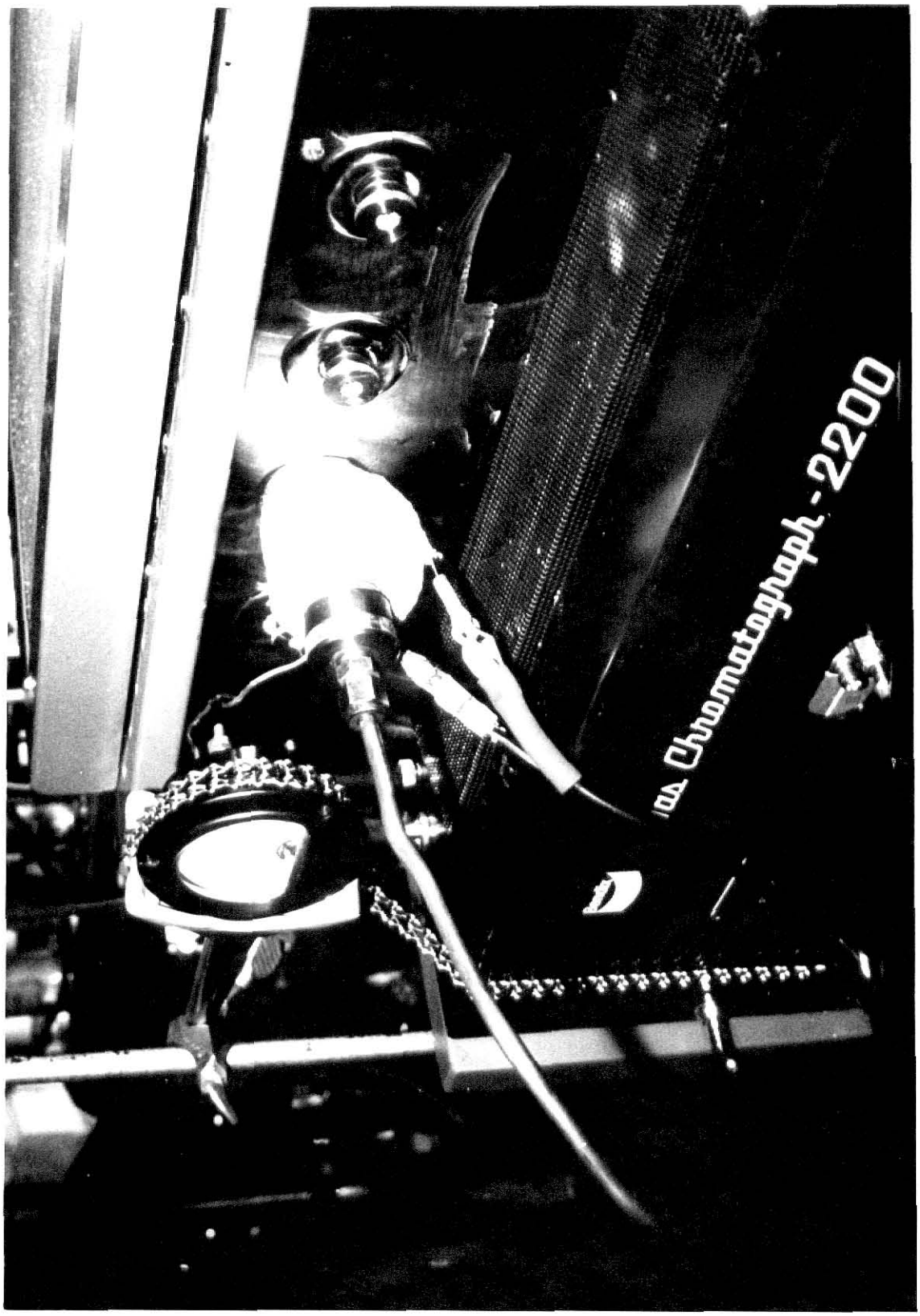
O--variac, 110 volt, for regulating heater current for injector


H
G
N
F
E
M
C
D
O
A
B
L
K
J
I

Photograph eleven; Assembly of apparatus inside the gas chromatographic oven.



Photograph twelve; injector assembly mounted on the injector port of the gas chromatograph.


Chromatograph-2200

olfactometer. It was found to give the results listed in the table below:

Fraction tested	Time of testing	No. of flies tested	Flies Attracted	Flies not responding
1	15 min	10	4	6
2	15 min	15	11	4
3	15 min	11	2	9

The third fraction was a control which was obtained by trapping column effluent on Tenax before the sample was introduced into the gas chromatograph. This was done to insure that any material trapped due to column bleeding would not give extraneous results due to fly attraction.

A sample of the odor obtained from 'paunch pellets' was taken in the same manner and tested under identical conditions. A gas chromatogram was not taken, however, the trapped odors being tested directly on the female flies. The results which were obtained from this test were:

No. of flies tested--27; no. of flies attracted--17; no. of flies not responding--10. This was done only for comparison purposes.

The process of trapping, chromatographing, and testing with flies of the separate halves of the chromatogram was repeated

Spectra Two; Chromatogram of cow manure odors trapped on Tenax-GC.

On this chromatogram and all subsequent chromatograms, the operating parameters are as follows:

Carrier gas--nitrogen

Column length--2 feet

Column diameter--3/16" O.D.

Flow rate:

Carrier--10 ml/min

Hydrogen--50 ml/min

Air--45 ml/min

Temperature:

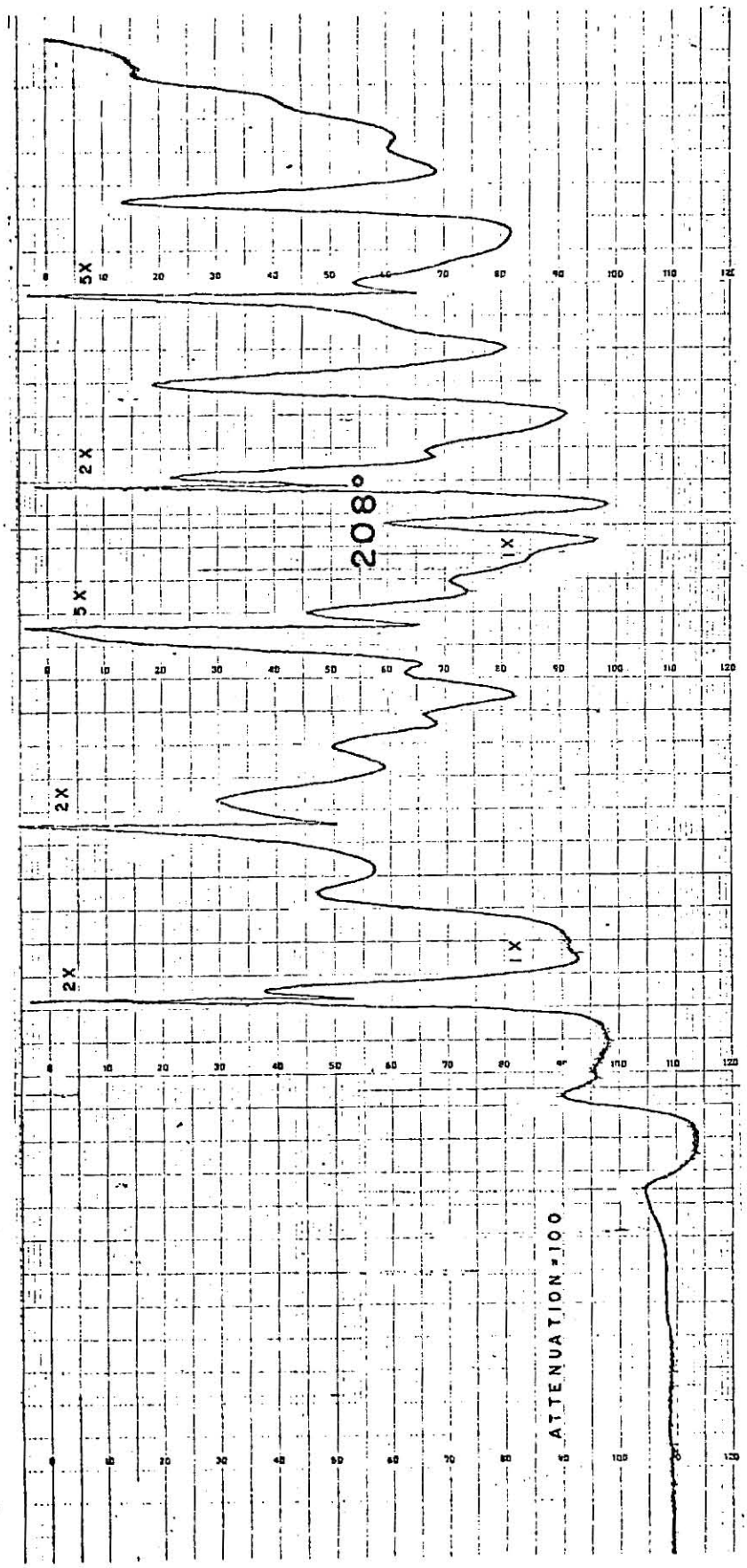
Injection port and detector--280^{o}C

Oven programming--6^{o}C per minute

Chart speed--½ inch per minute

Detector--flame ionization

Sensitivity--varies and is given on each of the chromatograms


208°
5X
2X
1X
5X
2X
1X
2X
ATTENUATION=100

twice more, but with very inconclusive results. It began to be apparent that the best conditions for the collection of manure samples was during the morning hours. All manure samples were obtained from bulls housed at the Artificial Breeding Station, Agricultural Extension Service, KSU. The bulls are maintained on a controlled diet, in which they are fed hay in the morning and hay plus grain in the afternoon and evening. A consultation with Dr. D. Upson, Veterinary Medicine Department, KSU, concerning the time for ingestion of the feed to elimination from the body of the animal, produced the information that this time would be, on the average, on the order of 100 hours. It has previously been stated that a hay diet produced manure which is fly-attracting. It was noticed that manure samples collected during the morning hours had a much greater attraction for flies than did manure samples collected in the afternoon. This is reasonable, in the light of the time required for digestion and elimination. Thenceforth, all samples were collected during the hours between 7:00 A.M. and noon, with much more consistant results. It was also found that certain bulls could not be used, as manure samples collected from them did not attract flies, no matter what time of the day the samples were collected. Another aspect of the manure collection which had decided results on the testing data was the moisture content of the sample. This was never measured quantitatively, but over a

period of time it became evident that if the manure sample when collected was very dry, it had very little attracting capacity for the flies.

All of these variables were taken into account when collecting the manure samples and it resulted in a much more reproducible chromatogram, so much so that when gas chromatograms of manure odors were compared from two different days, from two different animals, there is essentially no difference in them. This is shown by the two chromatograms on pages 57 and 58. Having determined this reproducibility, no attempt was made in future collections to obtain manure samples from the same animal. The only criteria being applied to the collection was the time of day and the moisture content of the samples, in a strictly qualitative manner. As mentioned, however, certain animals were not used.

The chromatogram was now divided into thirds, based primarily on temperature. These fractions were trapped after elution from the gas chromatographic column, as before, and tested on female face flies. The following results were obtained. All fractions were tested for a period of ten minutes.

Fraction	Total Flies	Sample Side	Blank Side	Unreacted
30°-150°	15	6	2	7
151°-240°	12	7	1	4
242°-305°	13	1	1	11

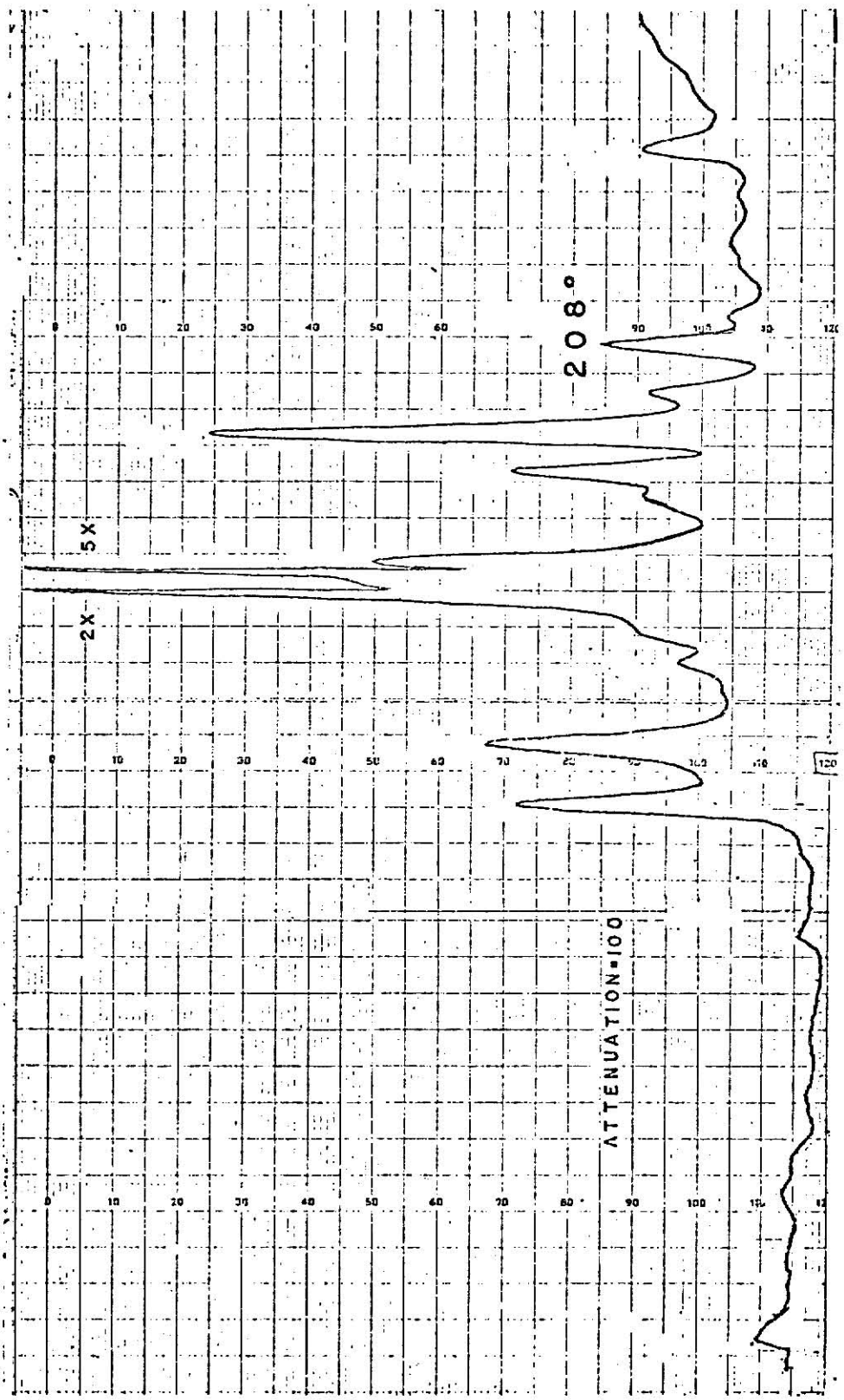
a second test was performed on a new sample in the same manner as above.

30°-150°	16	5	2	11
151°-240°	16	8	2	5
242°-305°	14	2	2	10

'Sample Side' and 'Blank Side' in the above table and in all of the tables following refers to the sample side of the olfactometer, containing the odors from the trapped samples, and the blank side of the olfactometer, containing only air passed through the reference side-arm flask. The reference flask contained Tenax, with no trapped material on it. This served as a control, so that any odor evolving from the packing material, as it was heated to drive off the trapped odors, would be the same for both arms of the olfactometer and so would eliminate any extraneous attraction of flies and thus affect the testing results.

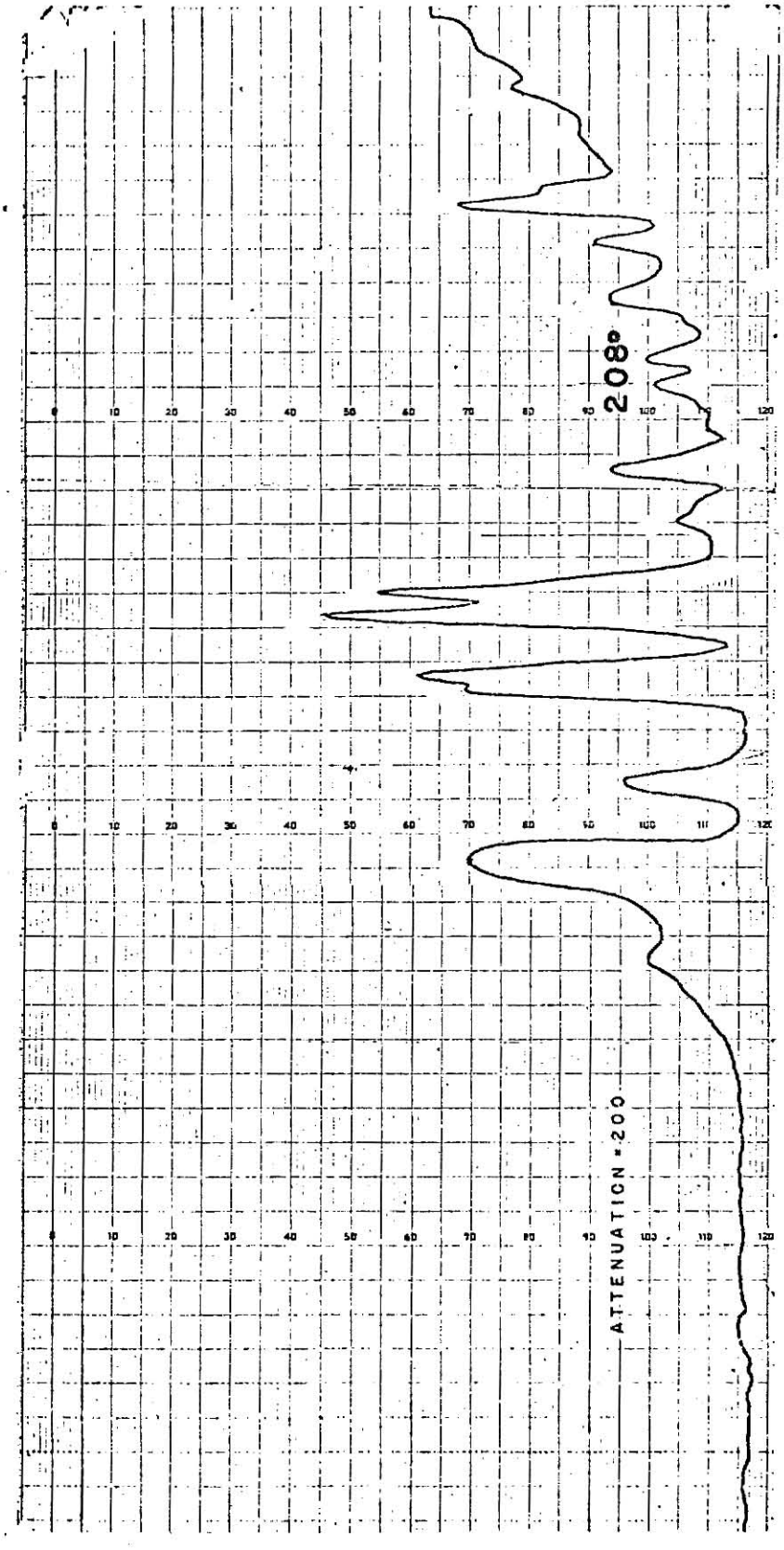
On the basis of the tests conducted thus far, it was concluded that the peaks eluting from the chromatographic column above a temperature of 240° are not significant and so this

Spectra three; Chromatogram of odors from a sample of cow manure


208°
5X
2X
ATTENUATION=100

Spectra four; Chromatogram of the odors from a sample of cow manure.

This chromatogram and some of those which follow, were reporoduced from the original through the use of a pantograph. The reason for this was that the original chromatogram, being recorded on pressure sensitive chart paper, was inadvertantly marked up and became unfit for use in this thesis. The originals were retained in the research file, however.


208°
ATTENUATION = 200

range from 240° to 305°C was disregarded in all subsequent trapping and testing.

A number of manure samples were collected and treated as before. The results of the fly tests are shown on page 59. In all cases the flies are tested first on fresh manure samples to insure that they are indeed responsive to the odor and can be attracted, that is, are ready to lay their eggs. In some of these cases, some batches of flies are more reactive than others and so the data is not always completely consistant. It was thought, however, that over a period of time and with the number of tests that were being conducted, that these discrepancies would average out, and that the results obtained would be a good indication of the attractiveness of the component peaks eluted from the gas chromatographic column.

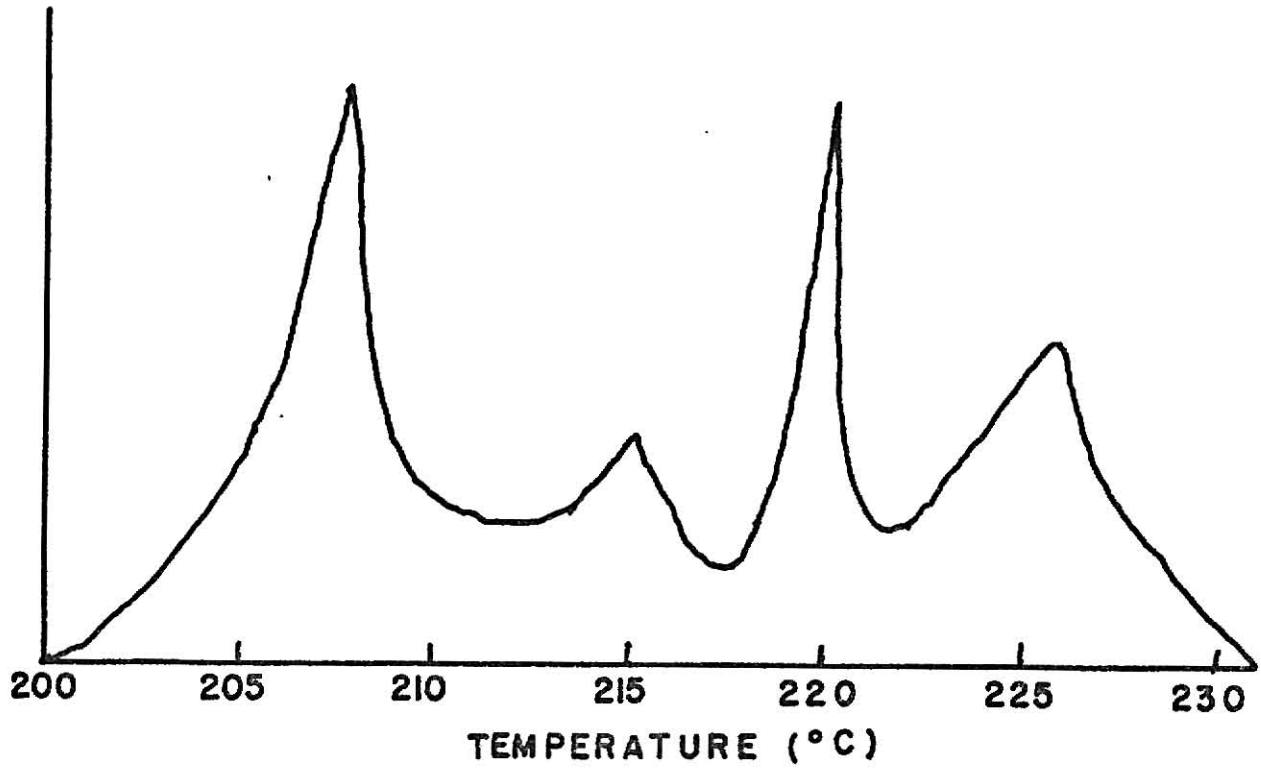
Sample	Total Flies	Sample Side	Blank Side	Unreacted
Test Number One				
120°-175°	16	6	3	7
175°-200°	13	5	5	3
200°-240°	14	11	1	0
Test Number Two				
200°-217°	15	12	2	1
217°-235°	19	9	0	10
Test Number Three				
200°-212°	18	5	0	13
212°-223°	13	7	0	6

The results of the tests as shown in the table above indicate that the attractant peak is in the range of temperature from 200°C to 240°C. The flies are attracted noticably faster toward those fractions collected near 200°C, on the programmed temperature chromatograms. Even though some of the flies are attracted to other fractions, it is always at a slower rate. The tests are not as conclusive as it was expected that they might be and so a study of the gas chromatograms from which the sample components were trapped was now done. This pointed up the following observation. The manure fractions which yielded only weak attraction for flies for any of the fractions have a different appearance than those which have a much stronger response. The assumption had been, in the past, that if the manure sample was collected during the morning, and certain bulls which were known to give poor results were avoided, that the samples would be consistant to a large extent. As seen from the general shapes of the two chromatograms given on the following page, this assumption was not a good one. These are representations of actual chromatograms, but are not the chromatograms, themselves. In all of the trials which follow, a chromatogram of each new manure sample was taken, before any of the fractions were trapped from that sample. If the gas chromatogram did not have the proper configuration, that sample was not used, and a new one obtained in its place.

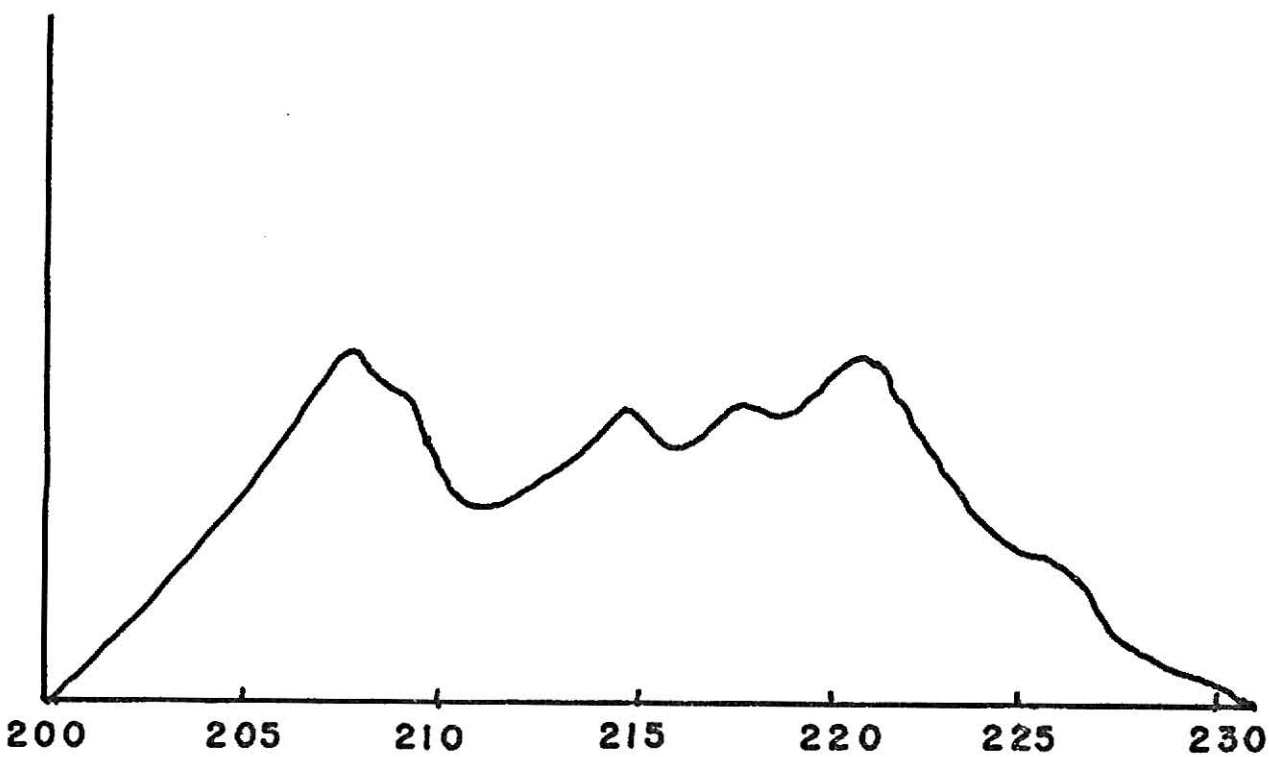
It was found that the column packing material, the Tenax-GC, was able to give good separation for approximately fifteen to twenty injections. The packing material then developed a 'memory' and would give extraneous peaks on samples injected after this time. This made it necessary to repack the columns after this period of use. It has not yet been determined how the best method of cleaning the packing material should be done. Heating the Tenax to 350^{o}C and holding it at this temperature overnight did not accomplish the removal of all of the residual components. Soaking the packing material in carbon tetrachloride did not serve the purpose either. The factory representative at State College, Penn., was contacted by telephone in order to determine whether there exists another method of cleaning the Tenax-GC. Since the packing material is new on the market, not much use has been made of it to date and no one else has had the problem of a similar nature because other workers were using more volatile compounds and lower temperatures. Therefore, no answer has been forthcoming, as yet. The problem seems to lie in the fact that other researchers have used the Tenax only as an adsorbant and not as a packing material for gas chromatographic columns.

Following the fabrication and conditioning of a new column, two new manure samples were collected, the volatile compounds

Figure nine; Gas chromatograms of a sample of the odors from cow manure, showing the configuration of the peaks from a 'good' sample and those from a 'poor' sample.



GOOD SAMPLE



POOR SAMPLE

trapped, and chromatographed to determine whether they were good samples or not. It was found that both were, so the following experiment was conducted. Since the chromatographic region of interest appeared to be that from 200°-240°C, it was decided that each of the peaks in this region, those being eluted at 208°, 215°, 220° and 230° would be trapped individually and tested for response with female face flies. This was done for both of the manure samples taken. The testing with flies would be done in order of elution with one of the samples, and then in reverse order with the other. This would then serve to determine whether the flies responded only to the fraction tested, or whether they responded to some residual odor from the fraction tested just previous to the one of current interest. The results of these tests are given in the table below.

Fly Response To Eluted Components

Manure Sample Number One. Tested in order of elution.

Fraction	Total Flies	Sample Side	Blank Side	Unreacted
205°-209°	21	16	1	4
210°-216°	17	10	0	7
217°-223°	16	5	2	9
224°-235°	16	5	2	9

Manure Sample Number Two. Tested in reverse order of elution.

Fraction	Total Flies	Sample Side	Blank Side	Unreacted
218^{o}-225^{o}	21	7	4	10
210^{o}-217^{o}	16	6	0	10
203^{o}-209^{o}	19	14	1	4
194^{o}-201^{o}	19	9	0	10

It can be seen from the tables above that the order of testing of the fractions had no effect on the response of the flies. Also it is evident that the portion of the chromatograms of greatest interest is that region around 205^{o}-216^{o}C, with the fastest and greatest response seeming to be to the fraction with the peak eluted at 208^{o}C. The slightly lesser response to the 215^{o}C peak was tentatively explained as follows: since the peak at 208^{o}C is large with respect to the one at 215^{o}C, it may tail into the latter peak as it passes through the chromatographic column, giving more positive results to the second peak than there actually should be. It had been repeatedly observed that the speed of response of the flies to the 208^{o}C peak was much greater than for the one eluted at 215^{o}C. In nearly all cases, the total number of flies to be attracted was accomplished in 90 seconds or less. Another possibility was investigated in the method of trapping of the eluted peaks. The apparatus used for this procedure is shown in the figure on the following page. Since the tubing leading to the U-tube containing the trapping Tenax was

not heated, it was thought that a likely source of cross-contamination of eluted peaks could be at this point. The eluted peaks would have time to partially condense on the walls of the plastic tubing used as this connector. Subsequent peaks could then wash this condensed material off of the tube walls and it would be trapped with a subsequently eluted peak. This could easily result in erroneous fly test results. A modification of the trapping apparatus was made. A 1/8" inside diameter stainless steel tube was substituted for the plastic tubing. A heating tape was wrapped around the stainless steel tubing and by connecting the heating tape to the electrical line through a variac, the tubing was heated to a temperature of 150°C and maintained at this temperature for the length of time required to elute all of the fractions of interest.

Another point in regard to the testing of the female face flies with the olfactometer needs to be considered. In tests conducted on a fraction which does not appear to be attractive to the flies, a random distribution of flies in both arms of the olfactometer should be observed. In some cases, this did not happen. Many of the tests which were conducted did give results in which the probability of random distribution of flies in both sides of the olfactometer was more readily apparent. This did not appear to be as consistant as it was

Figure ten; effluent trapping system used initially

A--gas chromatographic oven

B--electrometer

C--trap containing Tenax-GC

D--enlarged view of trap

F--outlet from gas chromatographic column

Figure eleven; effluent trapping system after modification.

A--gas chromatographic oven

B--electrometer

C--trap containing Tenax-GC

D--enlarged view of trap

E--variac to control temperature of heating tape

F--outlet from gas chromatographic column.

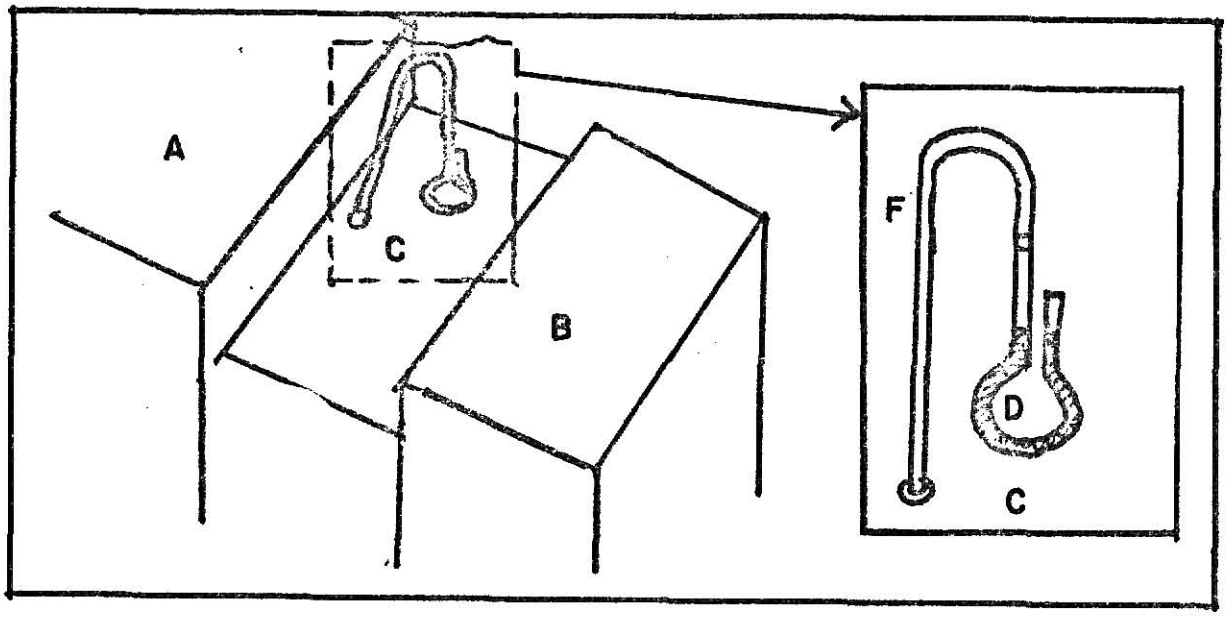


FIGURE 10

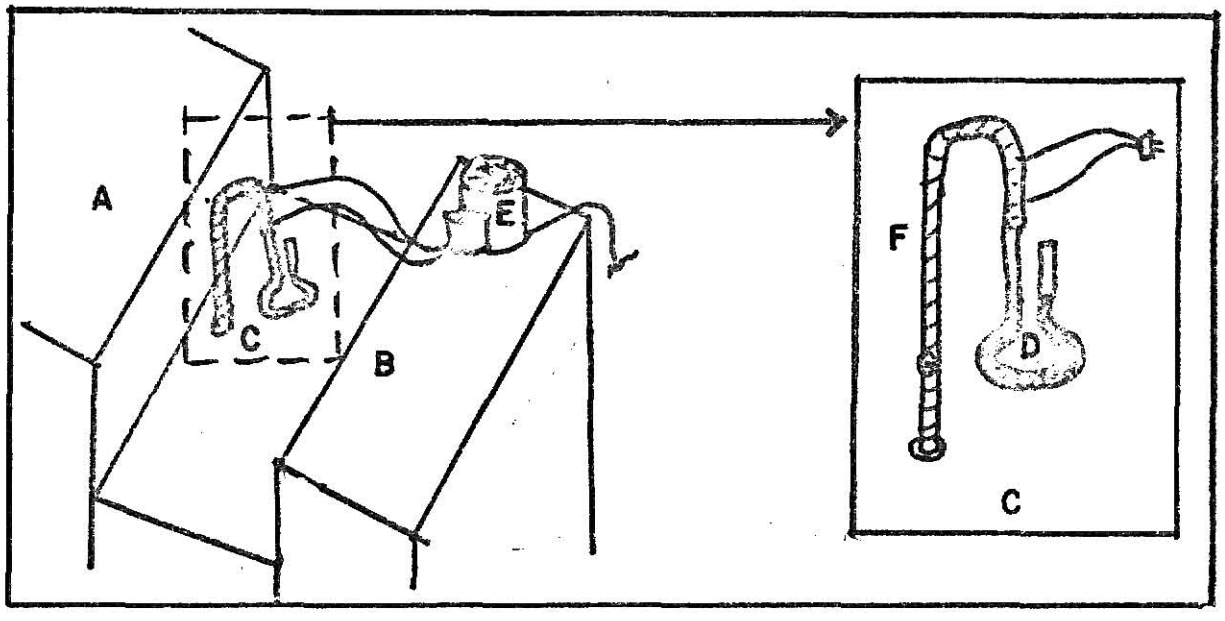


FIGURE 11

hoped that it would be. In order to better cancel out any residual effects of attractant components, the arms of the olfactometer were flushed with air after each test and then alternated between tests, so that the sample side during one test became the blank side during the following one. This method indicated that there was no residual attractive component left in the sample arm of the olfactometer, since the flies were not found to be in that arm at the conclusion of a test.

It has been shown, both in this project, and in other experiments conducted in the Entomology Department at Kansas State University, (Bay, 1974), that the flies themselves, are the best indicator as to whether a substance will attract the females or not. Since this is the case, an experiment was done to determine whether the component(s) present in the trapped volatile materials from cow manure could be identified in this manner. To accomplish this purpose, the experimental apparatus shown on page 68was constructed. A fresh manure sample was obtained, the volatiles trapped, and the sample odors then injected into the gas chromatographic column. It was postulated that a direct observation of the attraction of the flies in the olfactometer would allow the components of interest to be identified by correlation with the elution temperature as the sample was programmed through the column. The

Figure twelve; Experimental apparatus employed in an attempt to use female face flies directly as a detector of the component peak of interest by placing them at the column outlet.

A--gas chromatographic oven

B--electrometer

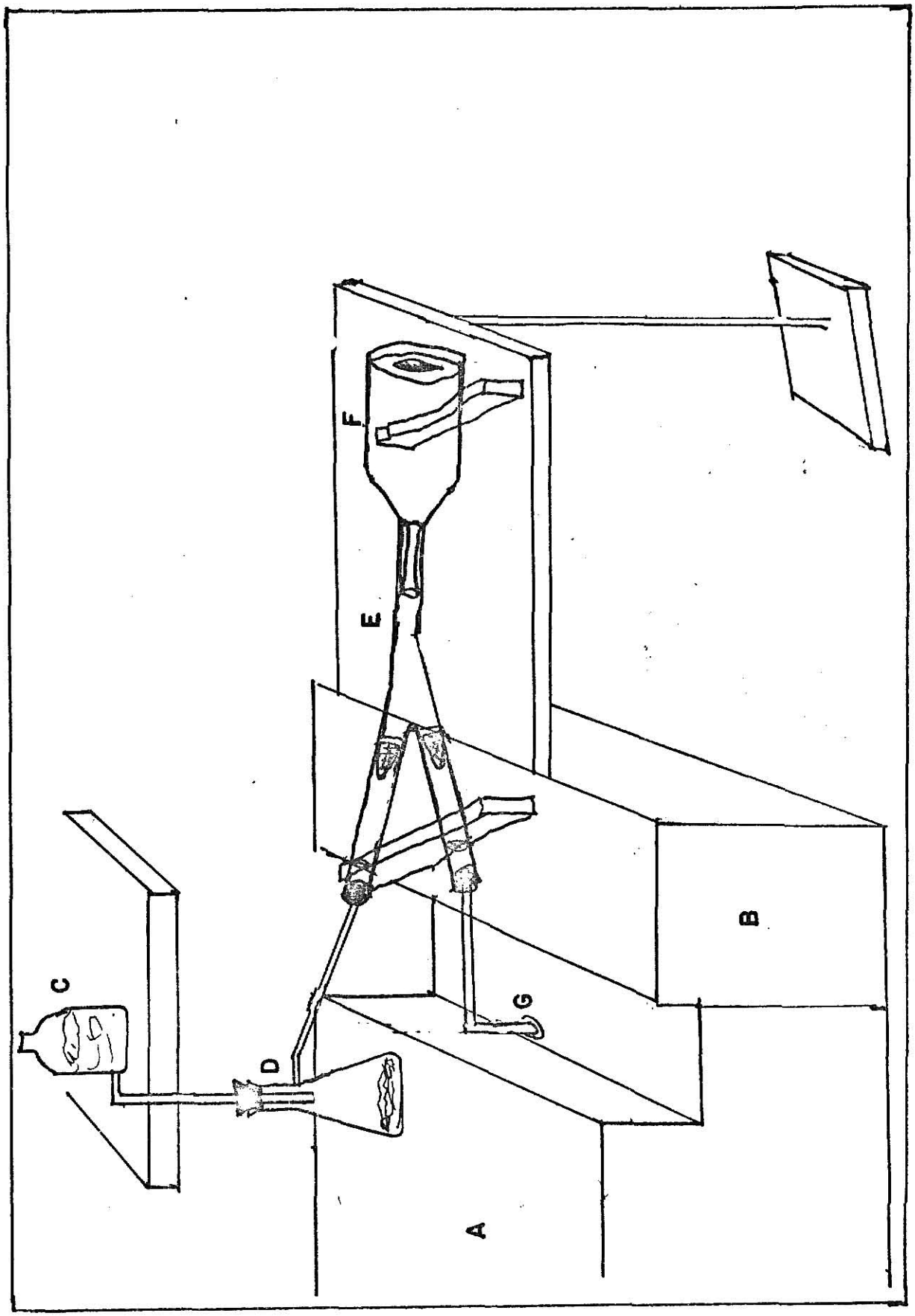
C--flask containing water used to force air through reference arm of olfactometer by gravity flow to a sidearm flask containing air (D)

D--side-arm flask containing air

E--olfactometer

F--reservoir for flies used in test

G--outlet from gas chromatographic column.

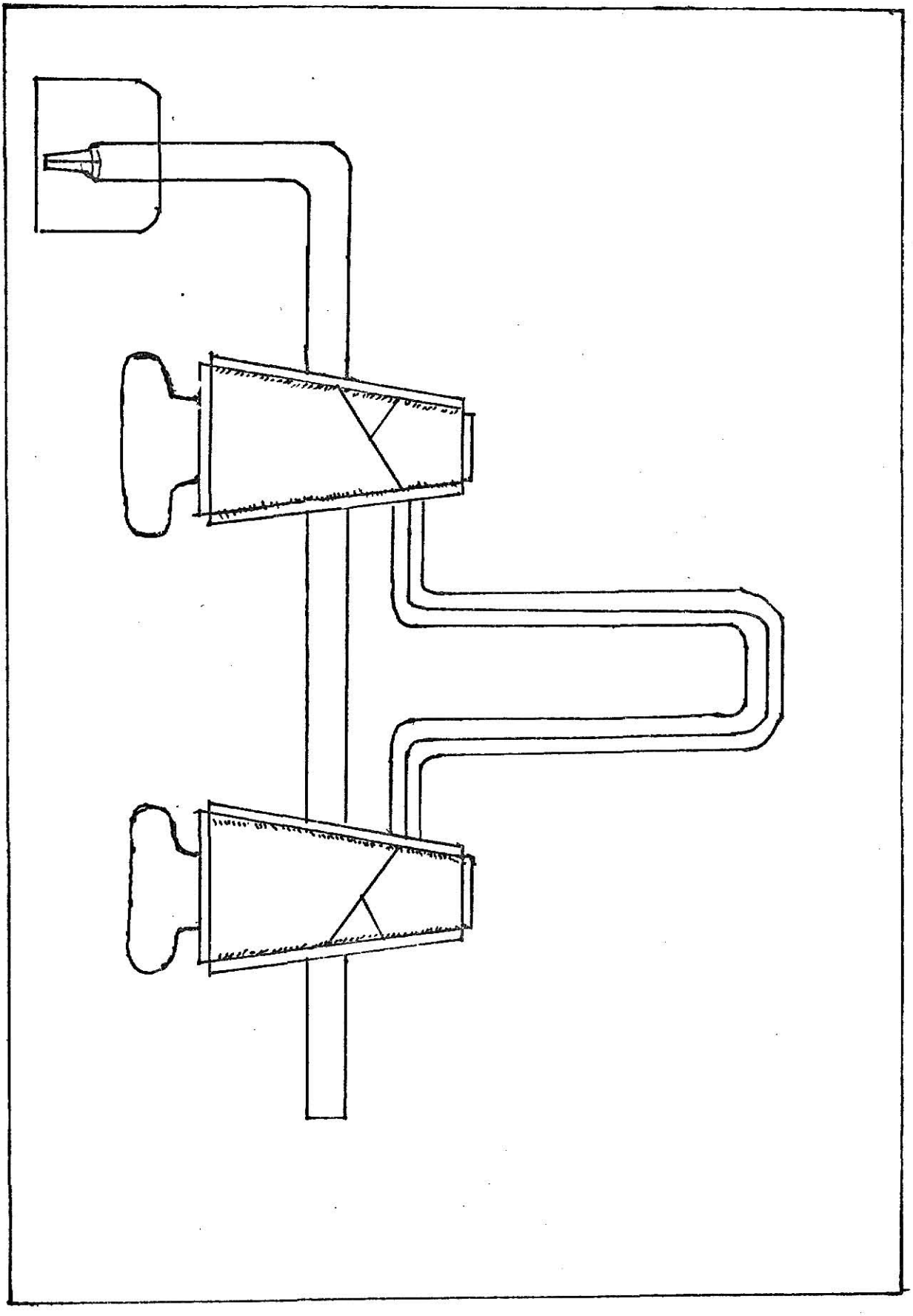

A
B
C
D
E
F
G

experiment did not produce any results. In fact, some of the flies expired. In assessing the cause of the failure of the flies to respond, the following explanation was given. In all experiments with flies as the detector, air was introduced as a carrier of the odor. The experiment performed as described above used nitrogen gas as the vehicle for transmission of the odor to the olfactometer. It is very possible that the lack of oxygen renders the flies totally unresponsive to any odor which might be present. This type of experiment had been successfully performed by other researchers on the gypsy moth. In these experiments, however, the moth had been attached to a support and located in the effluent stream from the gas chromatographic column, but in a room atmosphere, rather than a confined enclosure such as the olfactometer. Attempts at using the flies directly as a detector were abandoned in all further experiments for this reason and the effluent trapped on small portions of Tenax for introduction into the olfactometer.

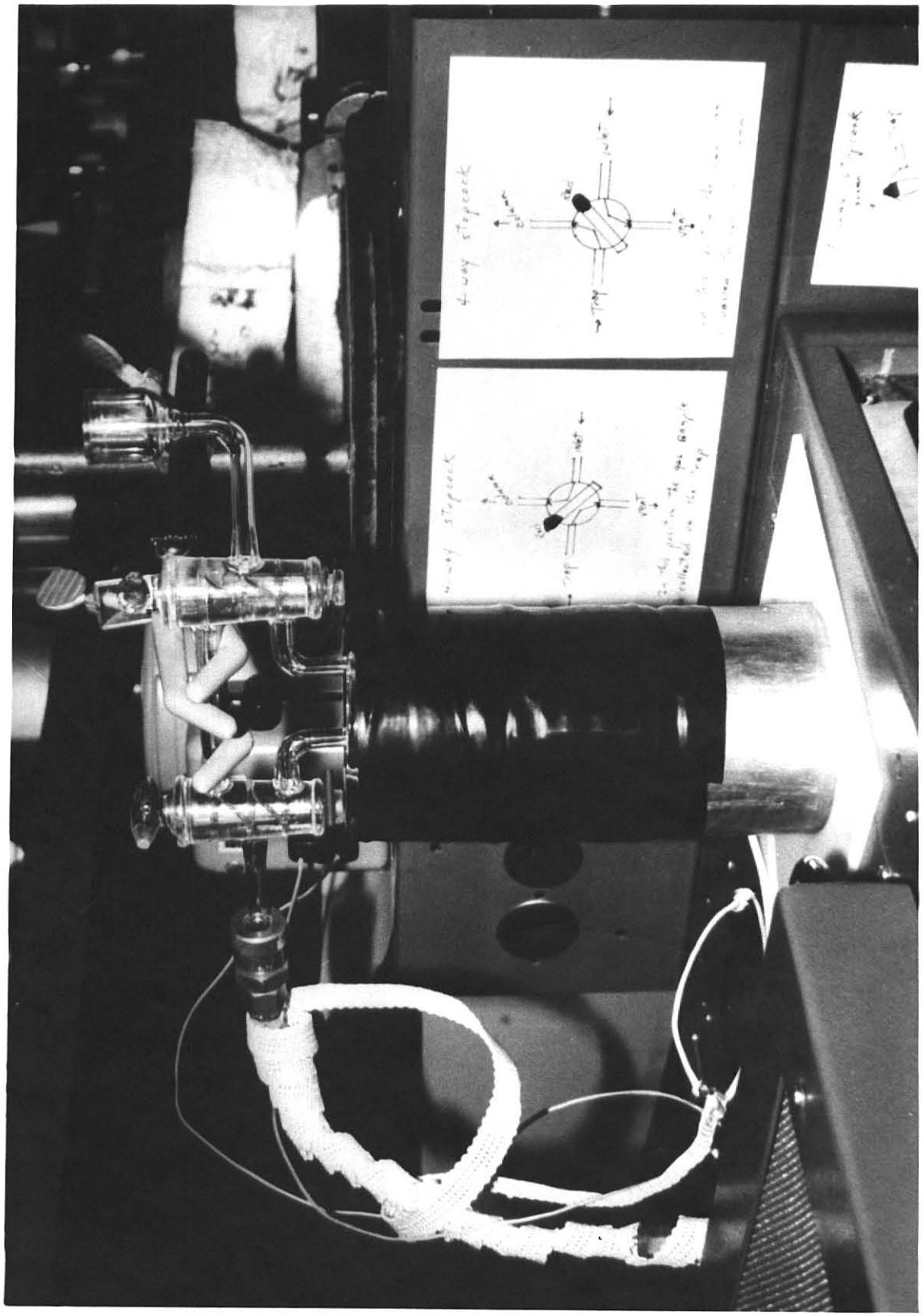
All of the information gathered thus far indicates that the peak at 208^{o}C is the attractant peak. It was desired that a mass spectrum of this component peak be obtained in order to attempt to identify the component, or at least try to determine whether it was a single component or a mixture of two or more components. A peak was trapped as it eluted from the gas chromatographic column through the use of the device

shown in the figure on the following page. This trapping device was fabricated by Mr. M. Ohno. The cup is the same as the one used on the liquid sample holder for an MS-902 mass spectrometer. The U-tube was made of heavy-wall capillary glass tubing, 1/4 inch outside diameter, so that it would concentrate the component in a small volume and render it easier to flush the peak component from the sampler into the sample chamber of the mass spectrometer. The two, three-way, glass stopcocks allow for the diversion of all component peaks eluting from the column until the one of interest, which is then directed into the U-tube. The U-tube is cooled in a liquid nitrogen bath during collection and kept in this bath until the component is ready to be flushed into the mass spectrometer. This transfer is accomplished by attaching the trap cup to the sample chamber inlet, evacuating the sample device capillary and warming the tube with one's hand. It was found that the use of a heat gun did not give any more improvement than did this method. The first mass spectrum obtained from a 208° component peak in this manner showed much fragmentation, with the molecular ion appearing at 511 m/e. This high molecular mass was viewed with some doubt, as the boiling point of the unknown constituent had been expected to be quite low, on the order of 100°-150°C. This assumption was based on the temperatures used in driving the component off the trap Tenax-GC. The component peak eluting

Figure thirteen; device used to trap column effluent for introduction into the mass spectrometer.



Photograph thirteen; apparatus for the collection of gas chromatographic column effluent for introduction into the mass spectrometer.


4-way stopcock
4-way stopcock

from the column immediately before the 208°C peak was then trapped by the same method and its mass spectrum taken. The two mass spectra were very nearly identical. This could indicate that either the two peaks were isomers of each other, or that one peak was contaminating the other, as they passed through the chromatographic column.

Since the results of the mass spectra indicated a possible contamination of the eluted peaks, a study was undertaken to determine the possible source of this contamination. The lubricant used on the stopcocks, both in the gas chromatographic oven and the mass spectrometer trapping device was Dow Corning Hi-Vacuum Silicone Lubricant. The mass spectrum of a sample of this lubricant was taken and found to be identical to those obtained from the component peaks earlier. This discovery had two very important implications. One, the lubricant had nearly completely masked the true mass spectrum of the component peak, and two, it also could account for the inconsistancy of the fly testing results. The stopcock lubricant could trap and hold a portion of a component as it is passing through the column and then allow a part of it to be bled off as another component passed through the column. This would most certainly produce cross-contamination and give the results observed for the fly reactions.

The entire glass system in the gas chromatograph oven was rebuilt, using new stopcocks, new glass tubing, and being lubricated with Apiezon M stopcock grease. The columns had been repacked with fresh Tenax. A sample of column effluent was trapped and its mass spectrum taken. The mass spectrum was found to show no background at all, other than a small amount of relative ion current due to a slight volume of air being introduced into the mass spectrometer along with the column effluent. The system was now 'clean,' and the work was resumed.

A fresh manure sample was collected, the volatile materials trapped, and the chromatographing carried out. The four eluted components of interest were trapped and tested with female face flies as before. The results of this test are shown in the table below:

Fraction	Total Flies	Sample Side	Blank Side	Unreacted
194^{o}-201^{o}	17	7	2	8
203^{o}-209^{o}	14	9	1	4
210^{o}-217^{o}	14	5	3	6
218^{o}-225^{o}	15	4	2	9

All tests were conducted for a time period of ten minutes.

While these results are by no means specific, they do show a little more discrimination between component peaks than the

Spectra five; Mass spectrum of Apiezon M Stopcock Lubricant.

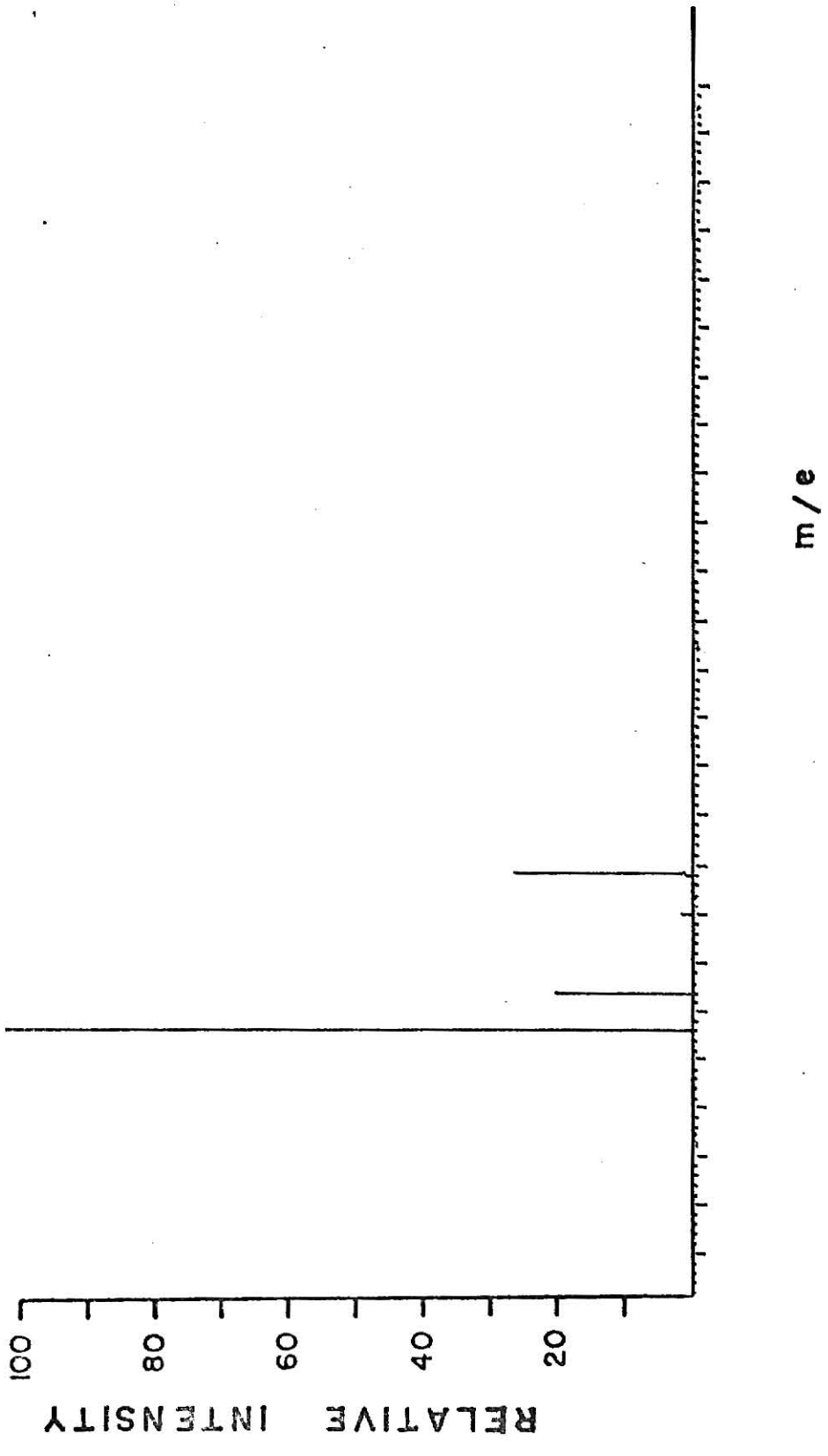
This mass spectrum and all of those following are plotted as relative intensity of ion current versus mass number. The relative intensity is on a scale of 100%. The m/e axis is calibrated as follows, each small tic represents an increment of one m/e unit, each longer tic represents an increment of 5 m/e units.

Instrument parameters

Electron impact mode

Ionizing voltage--70 ev

Source temperature--140^{o}C


RELATIVE INTENSITY
100
80
60
40
20
m / e

tests which had previously been conducted. The flies did not appear to be as responsive as usual and this may indicate that they were not quite ready to lay their eggs yet. It had been observed in the past in testing situations that this was sometimes the case.

The system now having been freed of the contamination due to the stopcock lubricant, another fresh manure sample was obtained and the process of trapping the 208°C peak for a mass spectrum repeated. It was found to be much less complex, with the molecular ion appearing at 106 m/e. A complete interpretation of the spectrum will be provided later. The appearance of a relative ion current appearing at 207 m/e was most puzzling, and a metastable defocusing experiment was performed on a new sample to determine whether this fragment had anything in common with a fragment at a lower mass number. It was determined that this component did not fragment to any mass number below it in the spectrum. It was concluded that this component is due to some compound bleeding off of the column packing material and does not interfere with the results obtained. This component can then be eliminated from consideration and does not appear in the mass spectra reproduced in these pages. It is known, through comparison, that it is not an active compound since commercially purchased attractant does not have it in its composition.

To ensure that the component peak trapped as it elutes from the gas chromatographic column is not contaminated by peaks before or after it, a fresh manure sample was collected and processed as before. The mass spectrum obtained was, with the exception of the intensity of a very few mass number relative ion currents, identical. This was interpreted, then, as being a pure component, although it still was not known whether this component eluting from the column may not still be a mixture of compounds not capable of being resolved by the chromatographic column packing material employed. This mass spectrometer experiment was repeated a number of times throughout the course of this research and the same results were always obtained. Mass spectra of the eluted peaks both before and after the peak of interest were taken and were found not to be the same as the mass spectrum of the 208°C component peak.

Dr. M. Hoffman, a faculty member of the Chemistry Department at KSU, and an expert in the interpretation of mass spectral data, was consulted with regard to the mass spectrum of the peak of interest. In his opinion, the compound would likely not contain any halogens, oxygen, or nitrogen in its composition. He indicated that it appeared to be aromatic and may contain a terminal methyl group. Since the molecular ion was seen to have a mass of 106 units, compounds such as ethyl

benzene, the xylenes, or related compounds became possibilities. Before work was done on these compounds, an additional mass spectrometer experiment was conducted, as discussed below.

A mass spectrum of the volatile component eluting from the gas chromatograph at 208°C from a paunch pellet sample was taken. On comparison with the mass spectrum of the 208°C component eluting from the gas chromatograph from a manure sample, it was seen to be different. It appears that the paunch pellet component is a mixture of approximately 50% of the same component as bovine manure and 50% of some other component. Further work with the paunch pellets was discontinued, having shown that the active attracting component was indeed present, no further use could be made of this type of sample without a separation or purification process being introduced.

The time consumed in trapping the samples into the oven of the gas chromatograph and then programming them through the column was of the order of two hours per sample. This limited the number of samples which could be handled in one day so it was thought that by removing the trap from the oven and operating the gas chromatograph in an isothermal mode, the number of samples would be increased in the same period of time.

Figure fourteen; experimental apparatus in which the oven trapping system was elimated, front view.

A--gas chromatographic oven.

B--outlet from gas chromatographic column

C--injection port

D--3-way stopcock

E--sample container and heater

F--purge gas inlet

G--vent

H--variac used to control heater temperature

Figure Fifteen; same system, top view, showing the configuration of the oven column.

A--gas chromatographic oven

B--Tenax-GC column

C--3-way stopcock

D--detector port

E--3-way stopcock

F--oven wall

G--sample container and heater

H--purge gas inlet

I--variac used to control heater temperature

J--vent

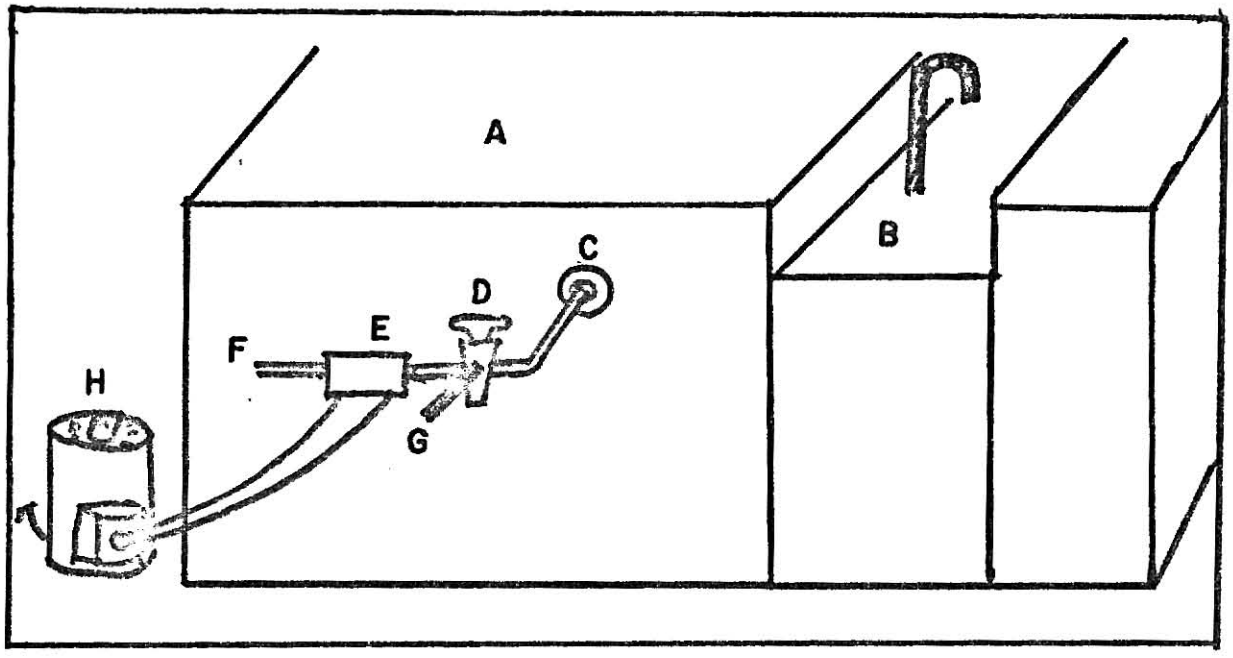


FIGURE 14

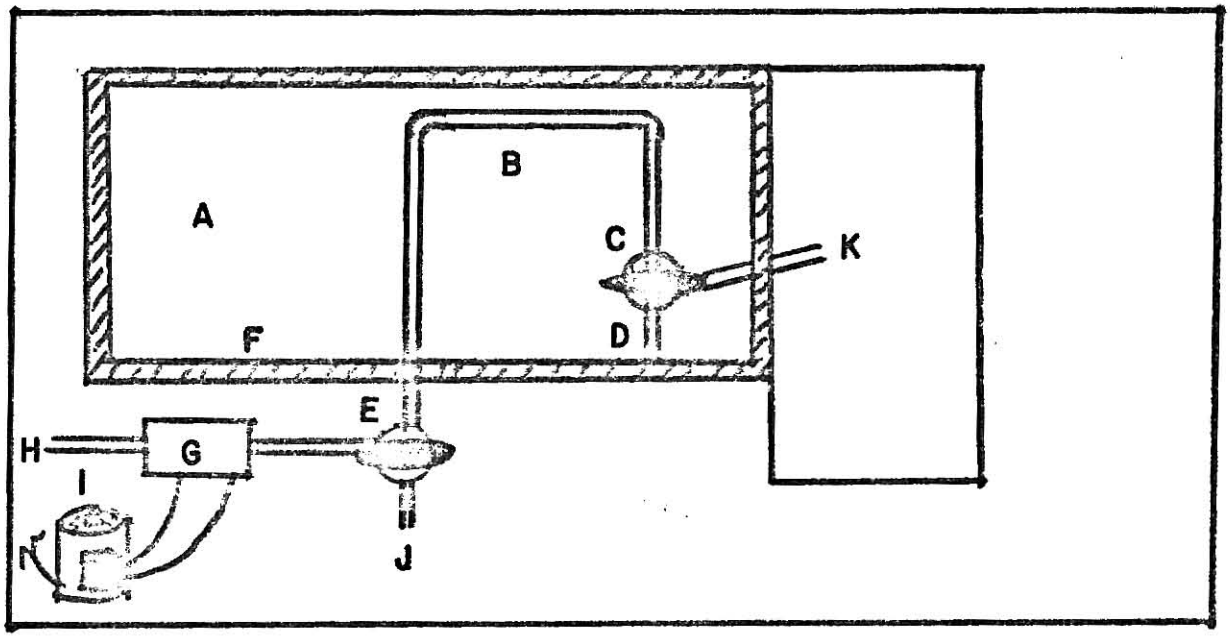
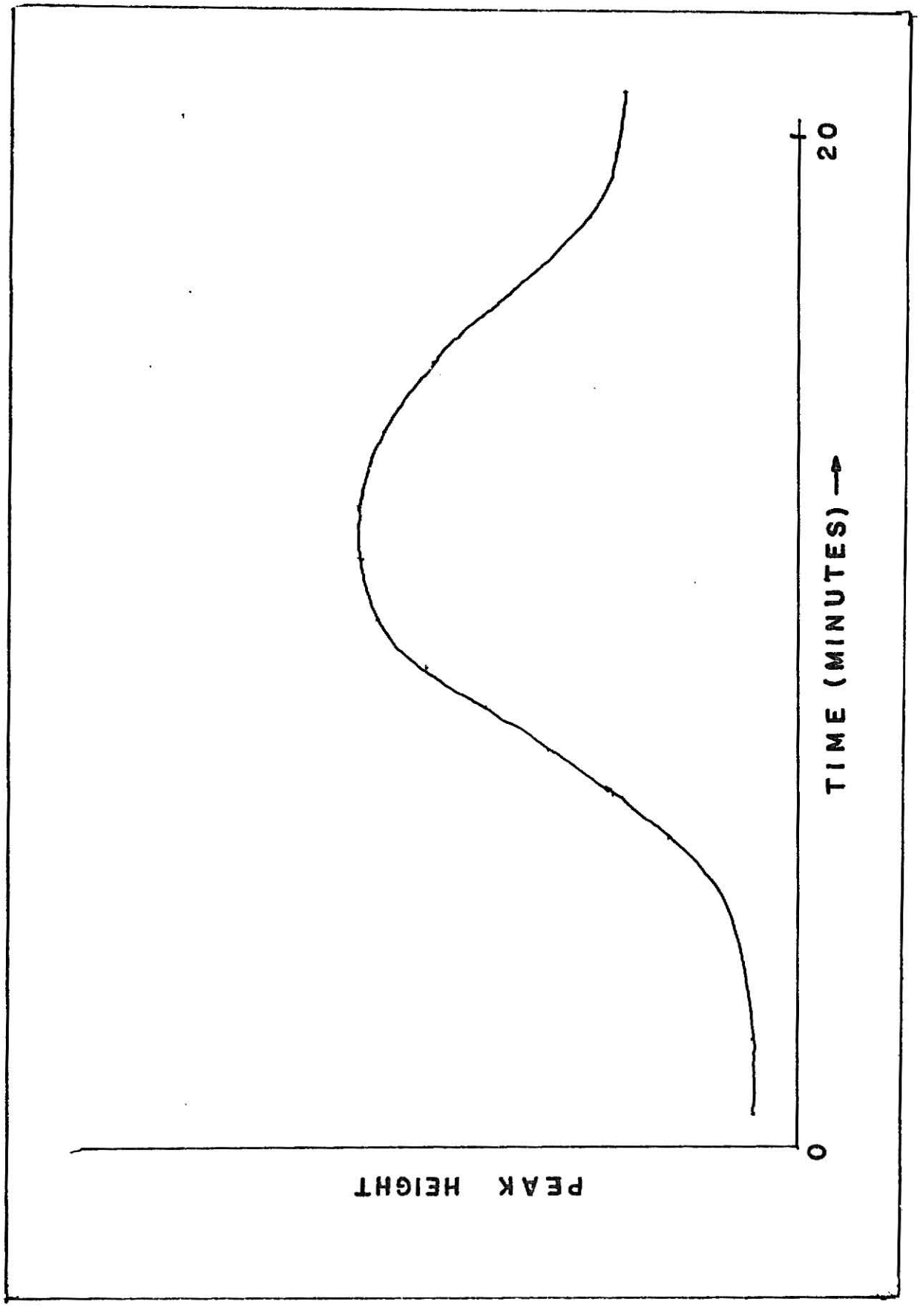


FIGURE 15

Figure sixteen; general shape of the gas chromatogram which was obtained using the system shown in figures fifteen and sixteen.


PEAK HEIGHT
0
20
TIME (MINUTES) →

This was then tried with the experimental apparatus being constructed as shown in the diagram on page 79. The newly modified system was conditioned and two fresh manure samples collected and chromatographed through its use. Both of the chromatograms which resulted had the general configuration shown on page 80 . It was apparent that operating the gas chromatograph in an isothermal mode was not going to produce any results, since the resolution of the component peaks was very poor.

The previous system, in which the 4-way stopcock is located inside the oven, and in which the gas chromatograph is operated in programmed temperature mode, was reassembled. After reconditioning the column, a fresh manure sample was collected and processed as before. A gas chromatogram was obtained for this sample to insure that the sample was indeed 'good' and contained the 208^{o}C component peak in a well-resolved quantity.

The mass spectrometer experiments were now continued in an effort to determine that the peak appearing at 106 m/e was indeed the molecular ion peak. A 208^{o}C was introduced into the mass spectrometer and a mass spectrum taken. It was seen to be identical with those obtained in the past. The energy of the bombarding electron beam was then reduced to 13 mev. This is the lower limit of the instrument used. This was

Table two; Data for mass spectra

Data For Mass Spectra

compound	mass number	relative intensity
208°C peak	39	8
	40	9
	41	6
	42	3
	43	10
	44	17.5
	51	8.5
	52	5
	54	1
	55	4
	56	3.5
	57	8.5
	63	4
	65	6.5
	70	3
	71	3.5
	78	5.5
	80	1
	91	35
	92	8.5
	103	5.5
	105	17
	106	19.3
	107	4.5

Data For Mass Spectra

compound	mass number	relative intensity
ortho xylene	39	6.9
	40	.3
	41	2.5
	42	.5
	43	23
	44	.1
	51	9.6
	52	4.6
	54	3
	55	2.4
	56	.9
	57	2.0
	63	4.0
	65	5.7
	70	.8
	71	.7
	78	5
	80	.5
	91	100
	92	7.7
	103	6.3
	105	26.9
	106	57.5
	107	5
	108	.2

Data For Mass Spectra

compound	mass number	relative intensity
meta xylene	39	7.1
	40	8
	41	1.1
	42	.1
	43	.2
	44	.1
	51	9.1
	52	4.9
	54	.2
	55	.1
	63	4.2
	65	5.5
	78	5.1
	80	.5
	91	100
	92	7.8
	103	6.2
	105	28.7
	106	61.7
	107	5.4
	108.0	.2

Data For Mass Spectra

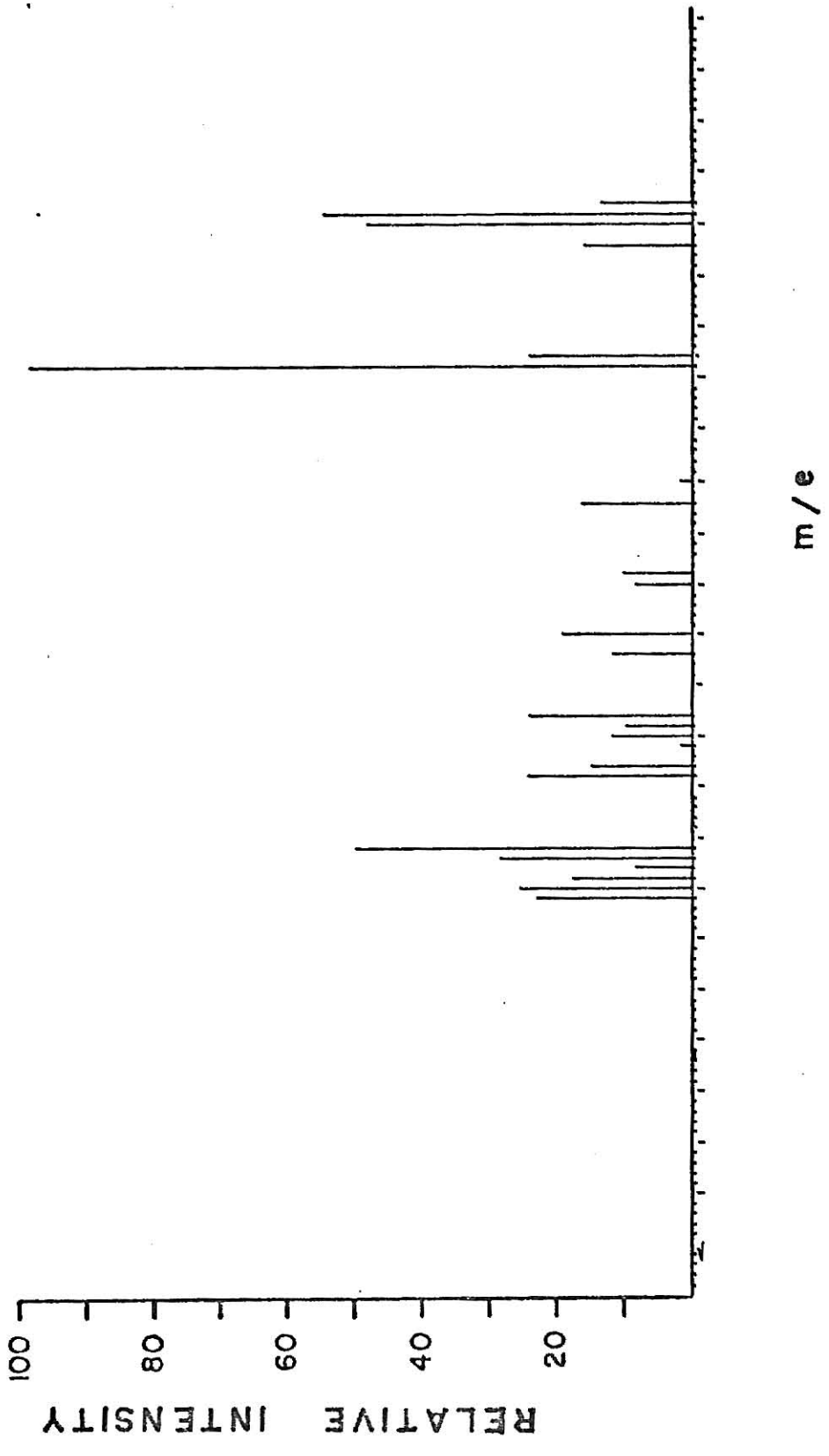
compound	mass number	relative intensity
para xylene	39	6.2
	40	.7
	41	1
	42	.1
	43	.2
	44	.1
	51	9.6
	52	4.6
	54	.2
	55	.1
	57	.1
	63	3.8
	65	5.3
	70	.1
	71	.1
	78	4.9
	80	.5
	91	100
	92	7.8
	103	6.4
	105	30.2
	106	61.1
	107	5.5
	108	.2

Data For Mass Spectra

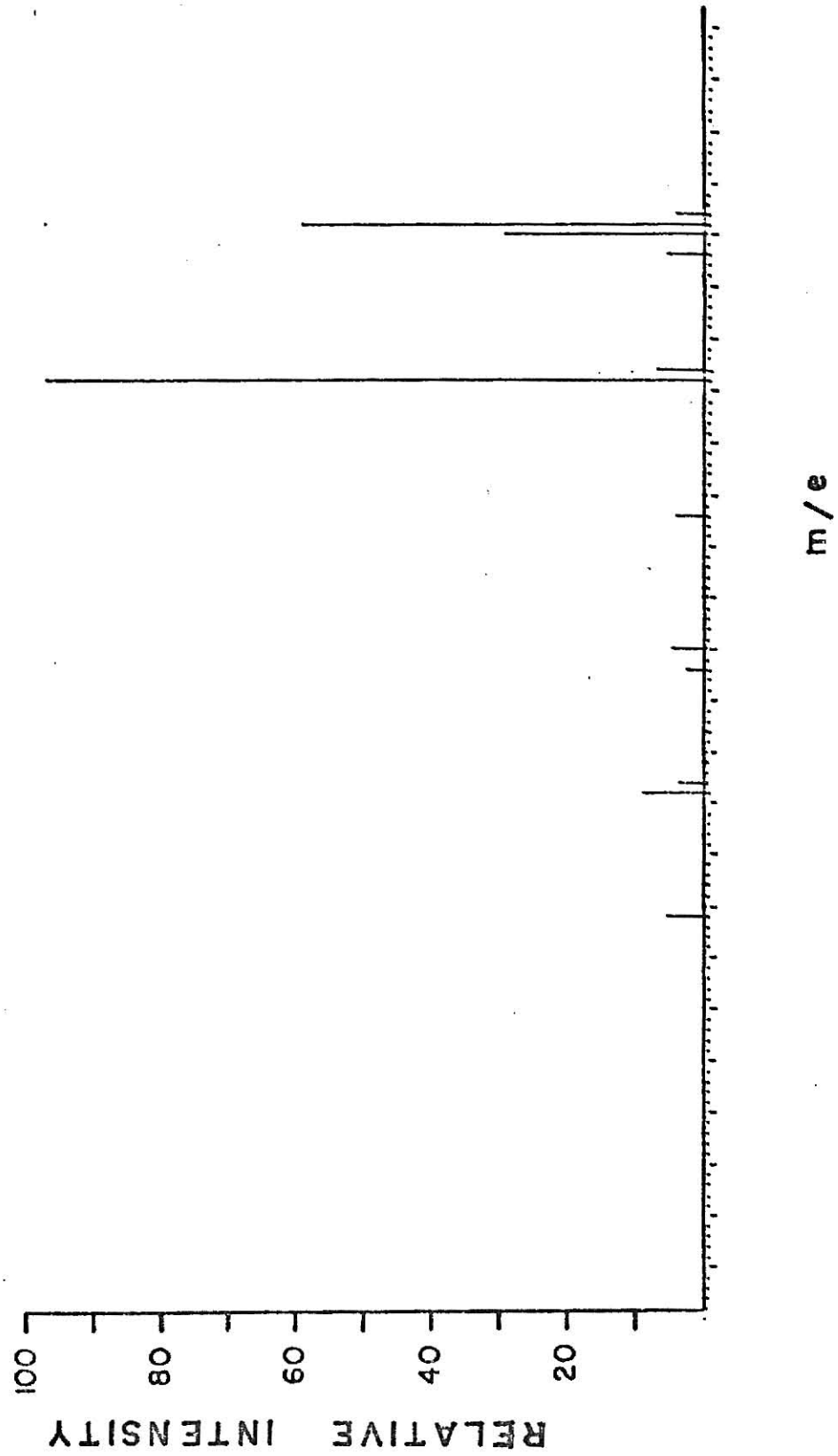
compound	mass number	relative intensity
ethyl benzene	27	6.3
	39	---
	52	13.1
	65	8.1
	77	7.8
	91	100.0
	92	7.5
	105	5.6
	106	30.6

done because it is known that if one reduces the energy of the bombarding electron beam to a value near the appearance potential of the molecular ion, the intensity of the molecular ion relative ion current will be increased, relative to the intensities of the fragmentation peaks. This, of course, depends on the stability of the parent ion. In general, aromatic, which was the class to which it was thought the unknown compound(s) belonged to, will give a prominent parent peak. When this was done, it was found that the peak at 106 m/e was indeed the molecular ion peak. Since there is a loss of 15 mass units in a major fragmentation from 106 m/e to the next large peak at 91 m/e, this is an indication of the presence of a methyl group. The strong peak at 78 m/e indicates the presence of the benzene ring. It was suspected that the identity of the compound may be one of the xylenes or ethyl benzene, as previously mentioned. The relative intensities of selected peaks on the mass spectrum for the unknown compound were measured and plotted. The literature was consulted for the average value of the relative intensities of the same peaks for the three isomers of xylene and for ethyl benzene. These plots and the data from which they were drawn are shown on the following pages. It can be seen from the information given, that the unknown compares more favorably with the meta and para isomers of xylene, than it does with the ortho isomer of benzene or ethyl benzene. This informa-

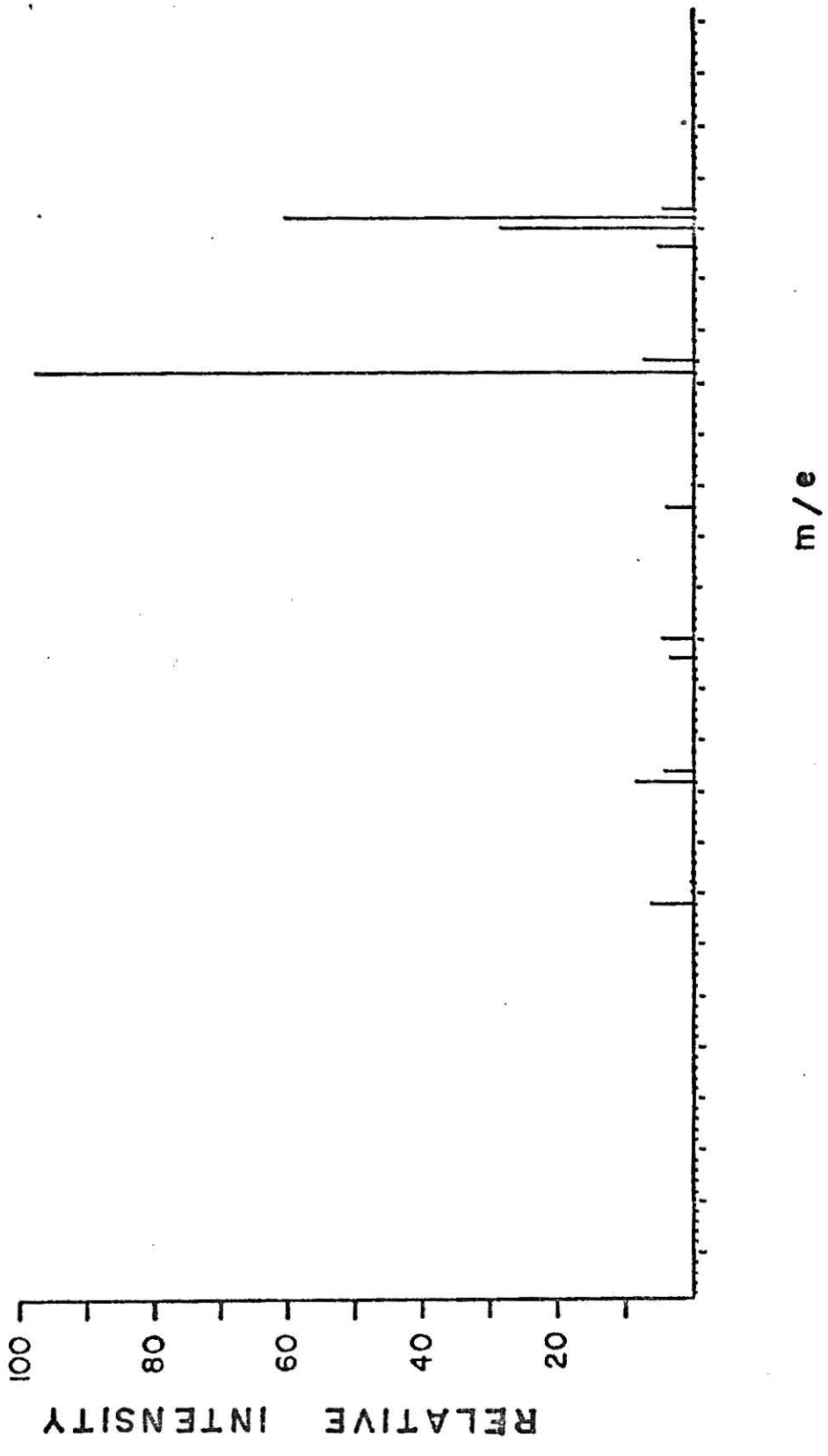
Spectra six; mass spectrum of the peak eluted from the gas chromatographic column at 208°C, a component of the odors trapped from a sample of cow manure.


RELATIVE INTENSITY
100
80
60
40
20
m/e

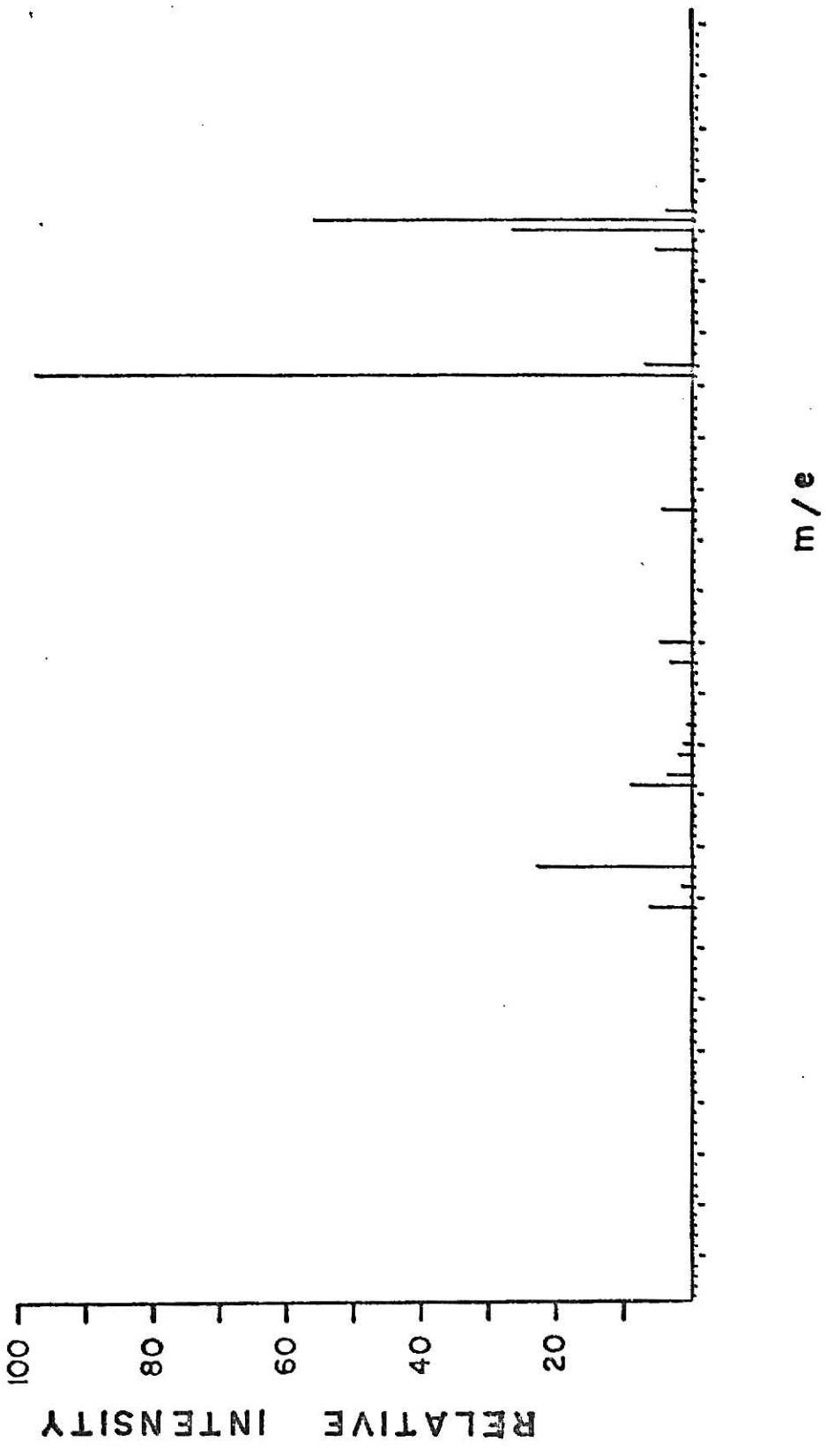
Spectra seven; Mass spectrum of the para isomer of xylene.


RELATIVE INTENSITY
100
80
60
40
20
m/e

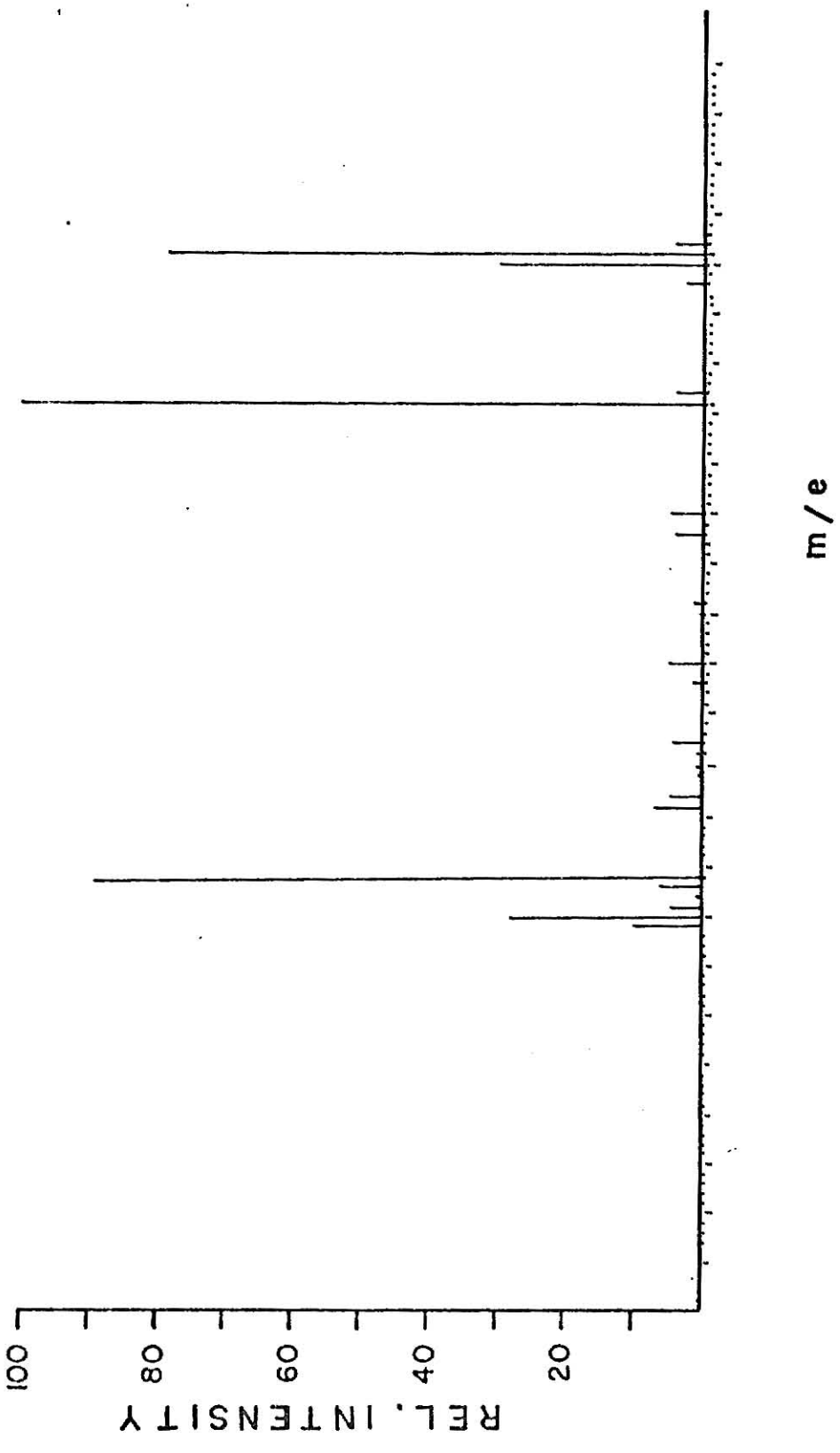
Spectra eight; Mass spectrum of ortho isomer of xylene.


RELATIVE INTENSITY
100
80
60
40
20
m/e

Spectra nine; Mass spectrum of meta isomer of xylene.


RELATIVE INTENSITY
100
80
60
40
20
m/e

Spectra ten; Mass spectrum of 208^{o}C gas chromatographic column eluted component from a sample of cow manure.


REL. INTENSITY
100
80
60
40
20
m/e

Spectra eleven; Mass spectrum of ethyl benzene.

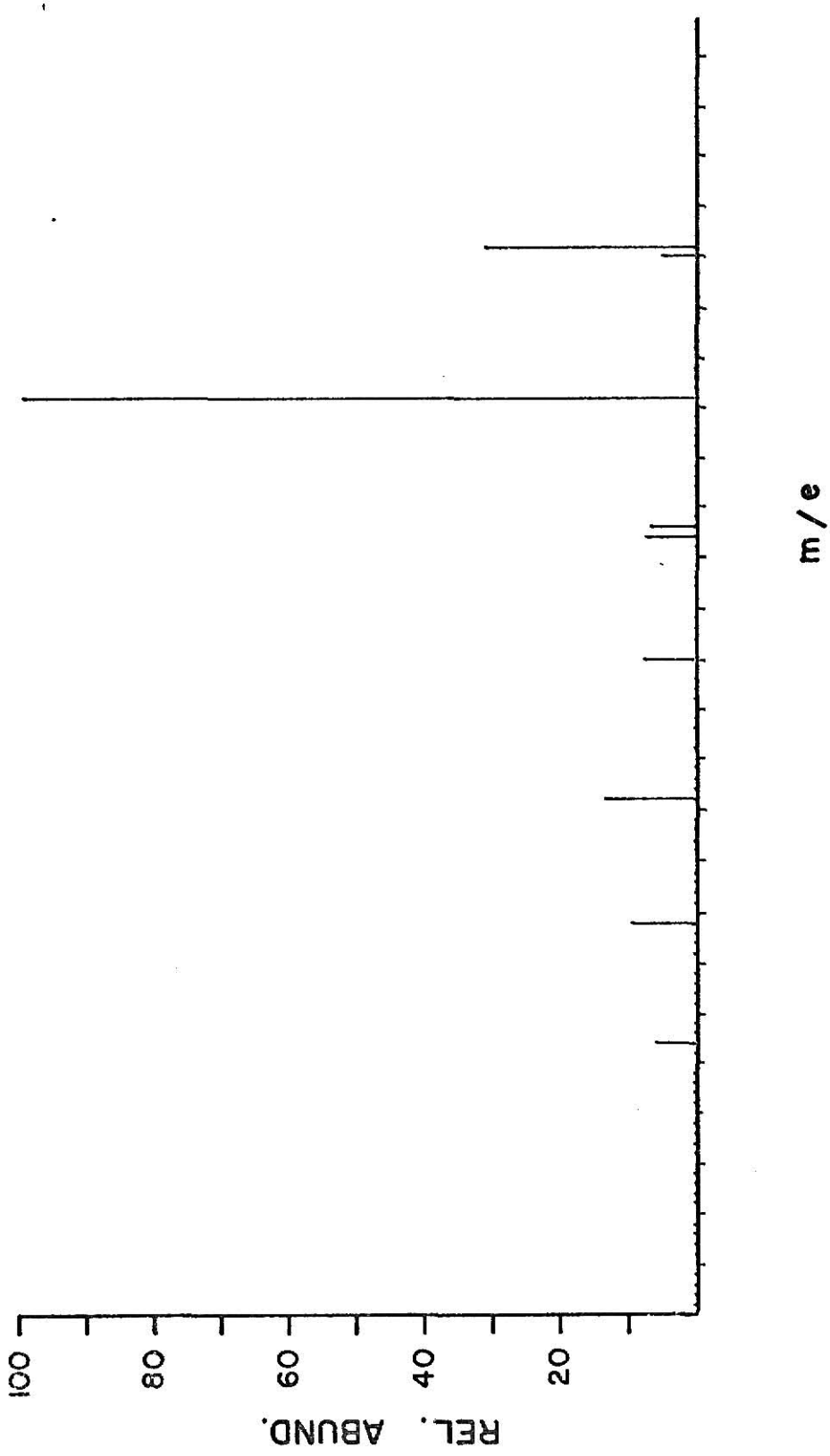

REL. ABUND.
100
80
60
40
20
m/e

Figure seventeen, device used to trap gas chromatographic column effluent for nuclear magnetic resonance spectra.

A--outlet from gas chromatographic column

B--Eight inch, Number 22, hypodermic syringe needle

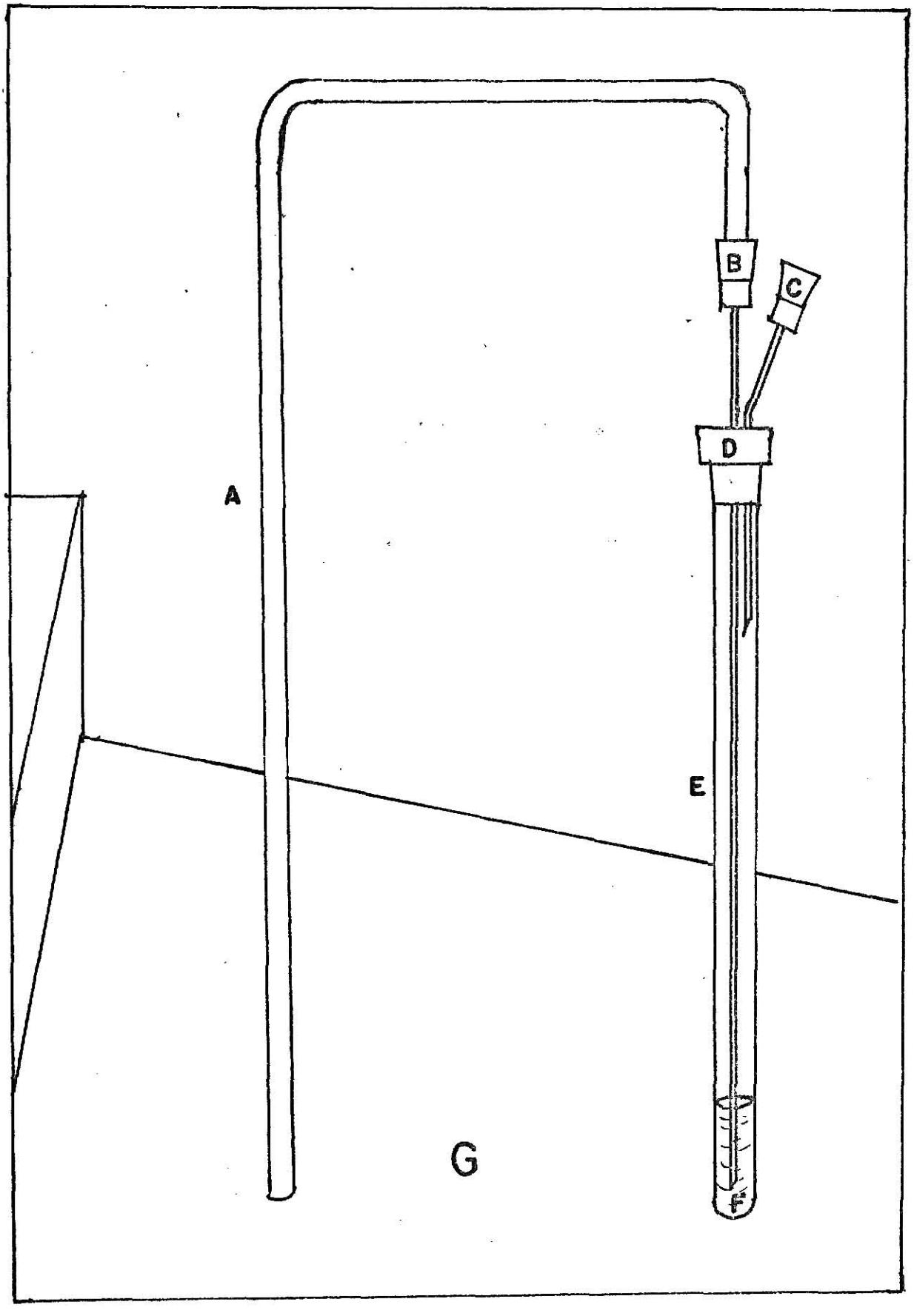
C--Two inch, Number 22, hypodermic syringe needle

D--septum cap for NMR tube

E--NMR tube

F--deuterated chloroform solvent

G--Electrometer


A
B
C
D
E
F
G

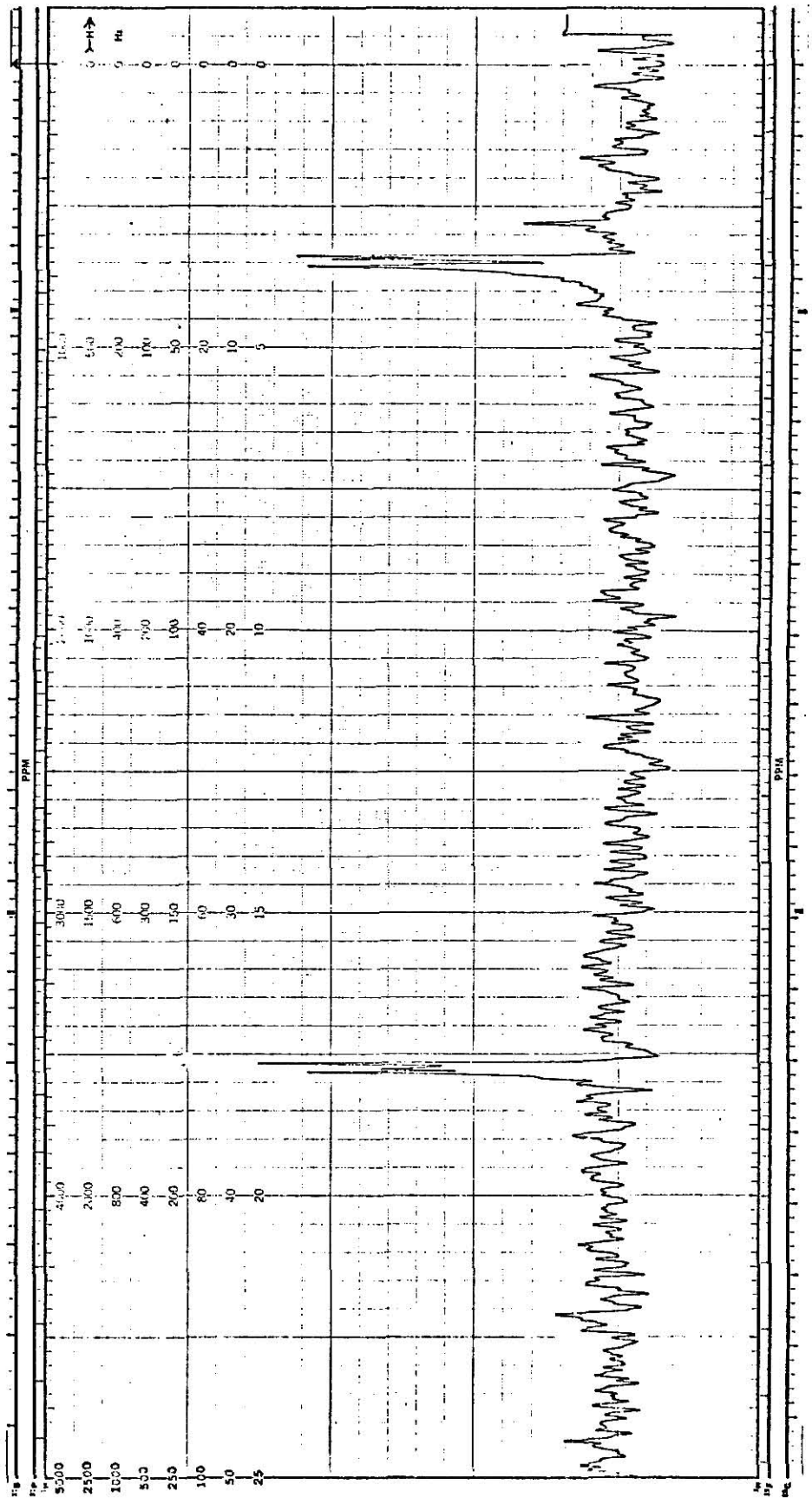
tion is not at all conclusive, however, it has only served to narrow down the possibilities of the identity of the unknown somewhat. It was proposed that an NMR spectrum of the unknown be obtained to better attempt to establish its identity. This was the next series of experiments undertaken.

A 208°C peak from a manure sample was collected as before, but the trapping device employed was modified. The diagram on page 90 shows this trap. The peak was allowed to bubble through deuterated chloroform in the hope that it would dissolve in this solvent. When an attempt was made to obtain a nuclear magnetic resonance spectrum of this sample, it was found that the concentration was too low to be able to allow for enough resolution for identification purposes. It was necessary, then, that a number of fractions of the component peak eluting at the desired temperature be trapped in a small volume of deuterated chloroform, reagent grade, highest purity. This was tried, with little success, due to loss of the component as it was being carried out of the solvent by the nitrogen carrier gas. The trapping system shown on the following page was then devised. The NMR sample tube was placed in an ethylene dichloride slush bath, producing a temperature of -36°C. The tube was capped with a rubber septum and an eight inch, number 22 hypodermic needle inserted through the septum to the bottom of the tube, containing 0.7 ml of

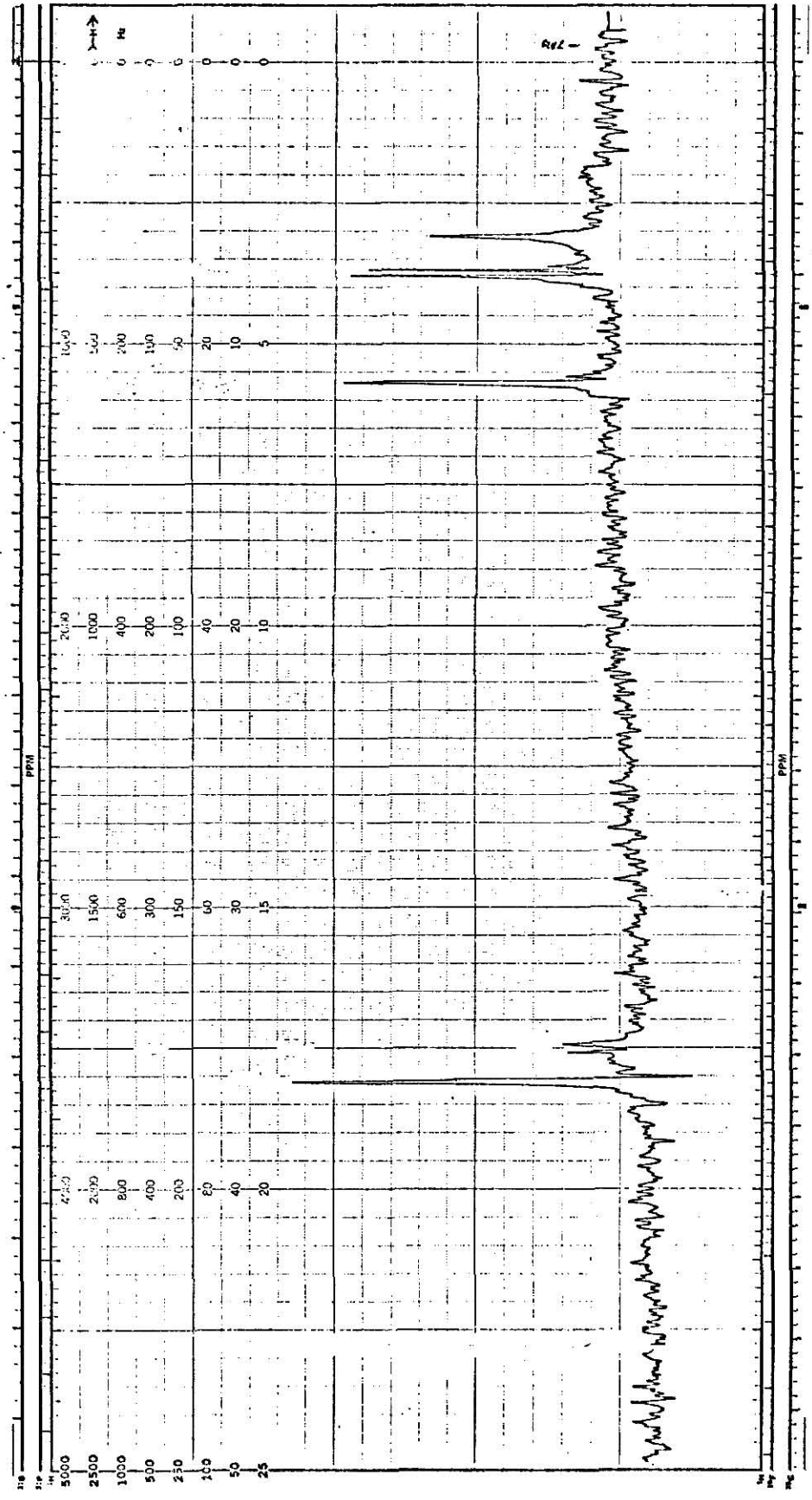
deuterochloroform. A two inch number 22 hypodermic needle was also inserted through the septum to provide a vent for the nitrogen carrier gas to escape, while restricting the evaporation of the solvent, should the necessity for this precaution arise. Thirteen, 208°C, component peaks were trapped as they eluted from the chromatographic column and the syring needles removed from the septum cap. The NMR spectrum was taken of the sample plus solvent, both as a single scan and as a time-average of nineteen scans, using an NMR, Model XL 100-15 . These spectra are seen on pages 94 and 95 , along with an NMR spectrum of the deuterochloroform, neat, on page 93 . Comparison of the literature spectra of the three isomers of xylene with that obtained from the NMR instrument, showed nearly identical signals between that of the meta isomer of xylene and the manure component peak. IF the small signals due to noise and to 'glitches' from the time-averaging device are ignored, measurement of the signals due only to the sample, disregarding those signals known to belong to the solvent, produces a chemical shift of 468 for the unknown, as compared to a chemical shift of 470 for the known meta isomer of xylene. The literature spectra are shown on page 96 for comparison.

The boiling points of the three isomers of xylene are sufficiently different to allow for separation on a gas chromat-

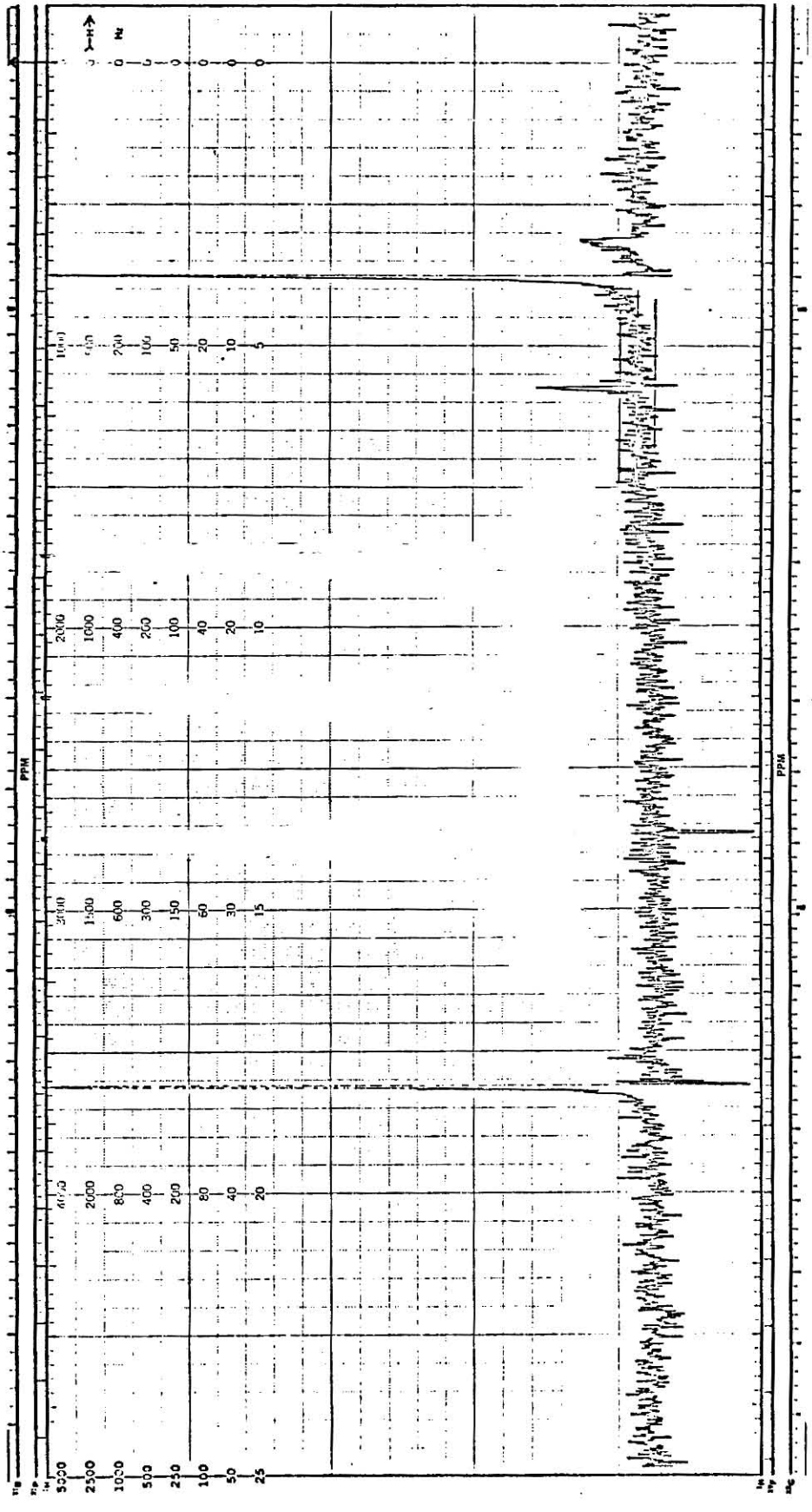
Spectra twelve; NMR spectrum of deuterated chloroform solvent.


PPM
PPM

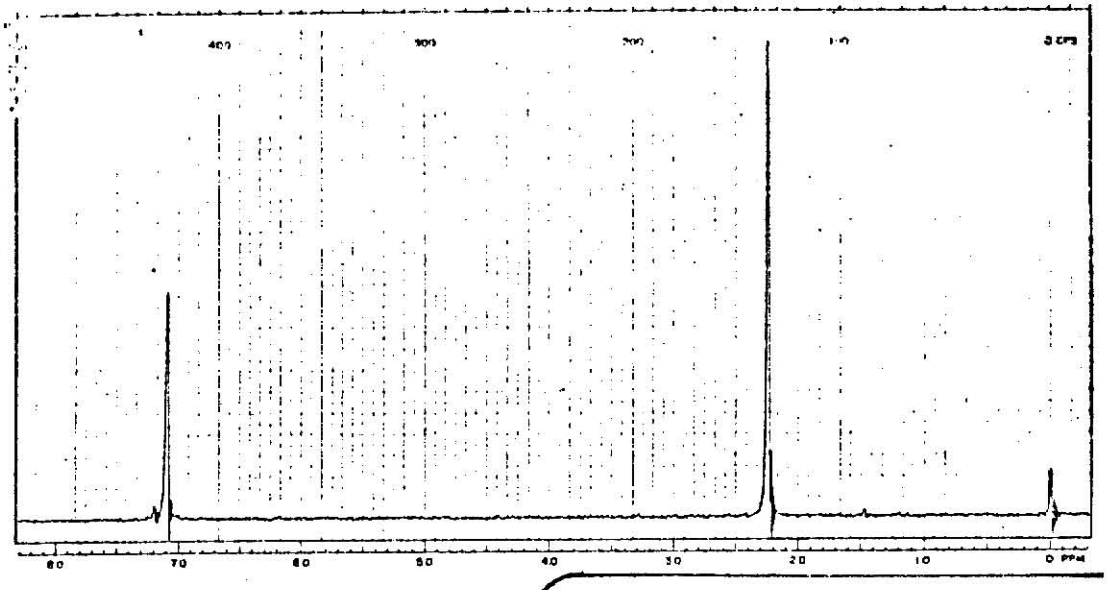
Spectra thirteen; NMR spectrum of deuterated chloroform solvent plus the attractive peak eluted from the chromatographic column at 208°C. Time average of 19 scans.



Spectra thirteen (b); NMR spectrum of deuterated chloroform solvent plus the attractive peak eluted from the column at 208°C. One scan.

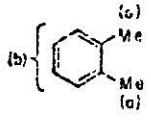

PPM
PPM

Spectra fourteen; Literature NMR spectra of the ortho, meta, and para isomers of xylene.



o – xylene

C_8H_{10}



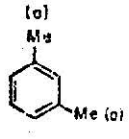
ASSIGNMENTS

Sweep offset: _ – _ ppm
Freq. response: 2 cps
Sweep time: 250 sec
Spec. amp: 8.0

a 2.25
b 7.10
c
d
e
f
g
h

m – xylene

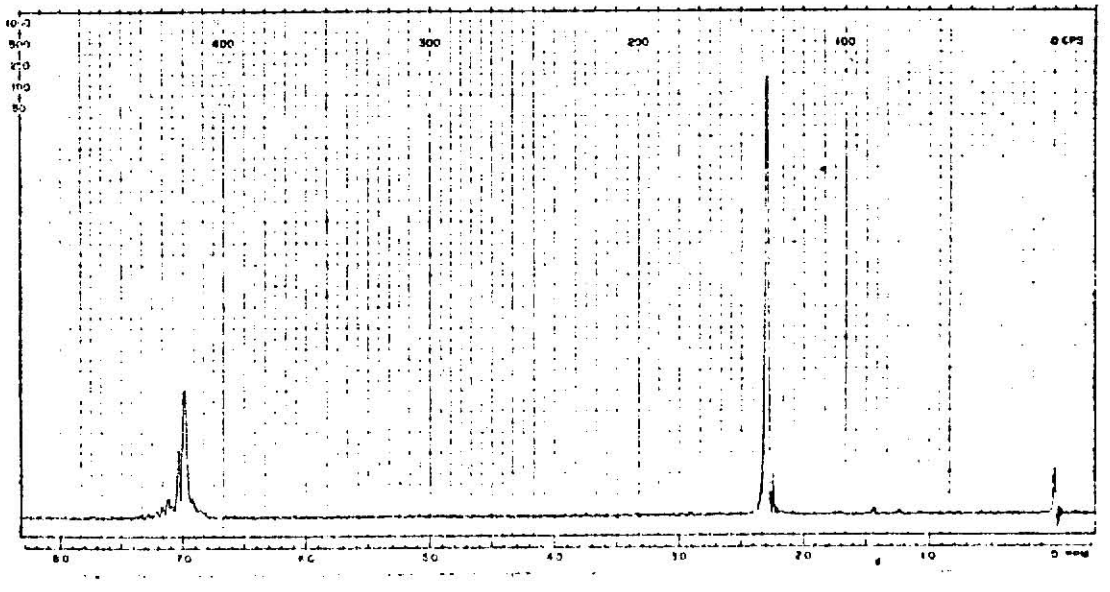
C_8H_{10}

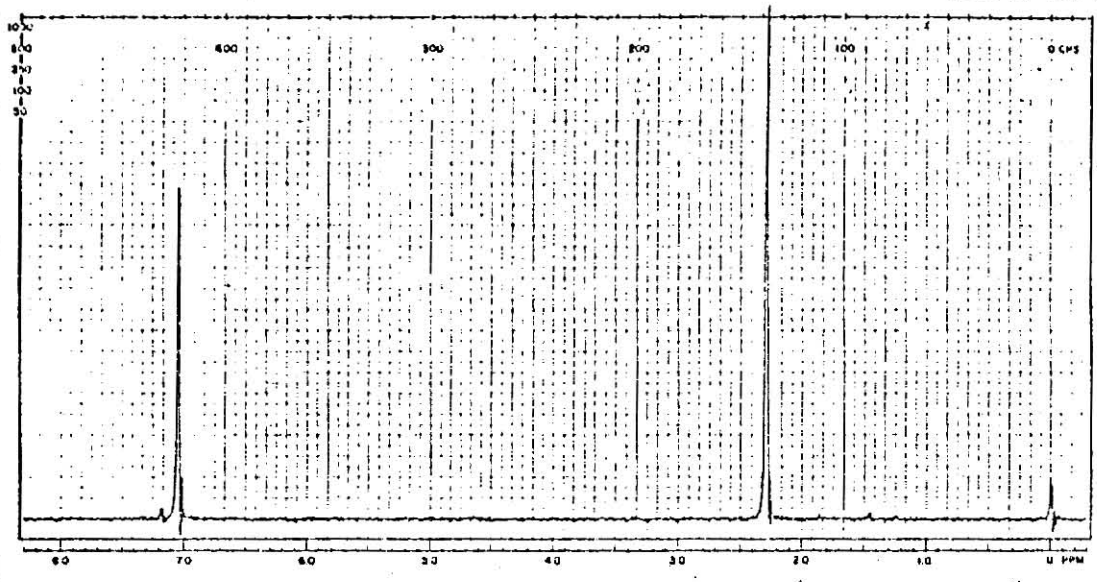


ASSIGNMENTS

Sweep offset: – ppm
Freq. response: 2 cps
Sweep time: 250 sec
Spec. amp: 8.0

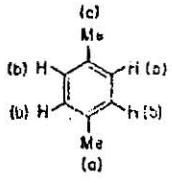
a 2.30
b
c
d
e
f
g
h





p – xylene

C_8H_{10}



Sweep offset: ___ ppm
Freq response: 2 cps
Sweep time: 250 sec
Spec amp: 8.0

ASSIGNMENTS

a 2.30 e ____
b 7.05 f ____
c ____ g ____
d ____ h ____

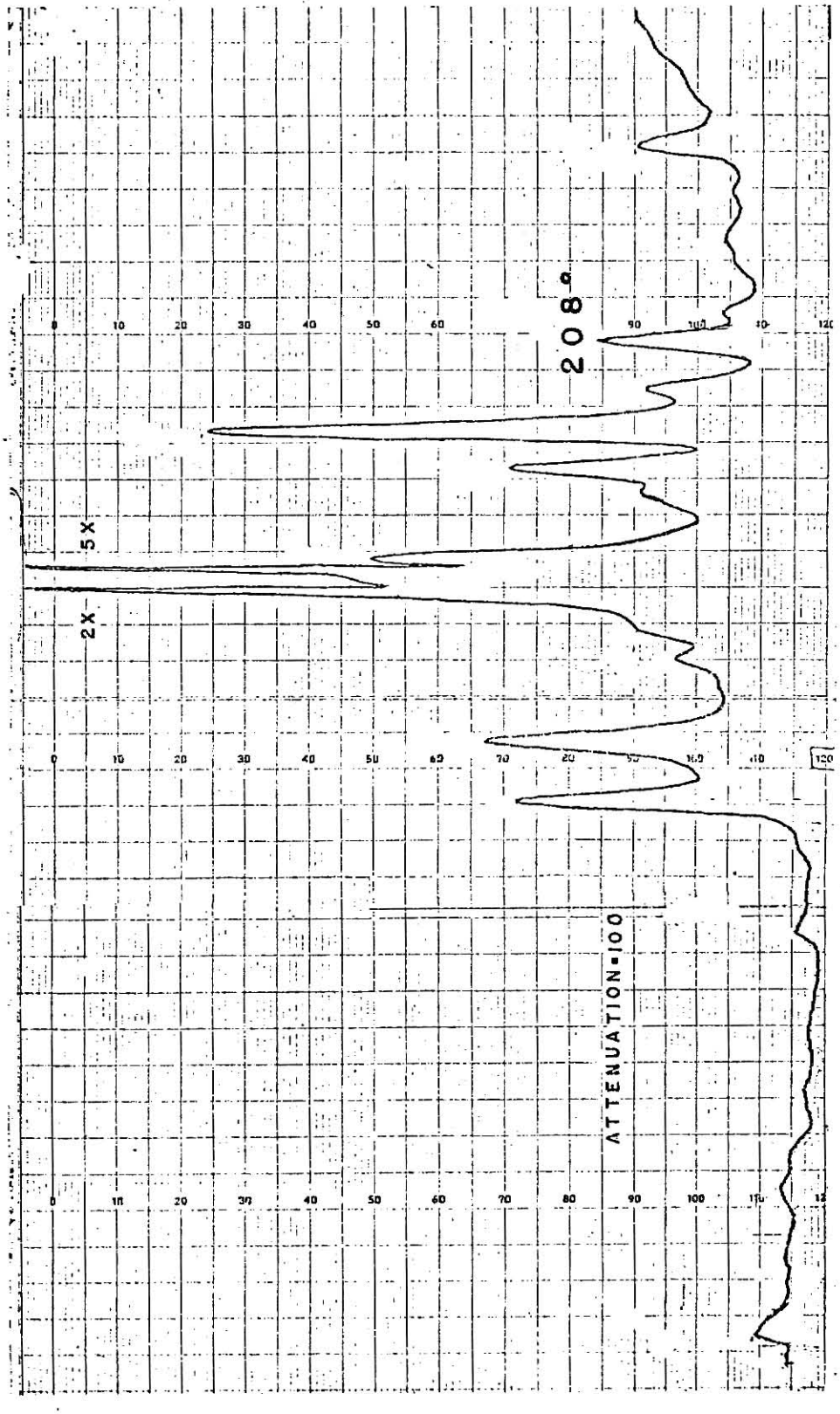
ograph equipped with a flame ionization detector. In order to further attempt to establish the identity of the component peak from the manure samples, the following was done. Three samples of the odor from cow manure would be 'spiked', each with a different isomer of xylene, and chromatographed as before. The sample containing the proper isomer of xylene would show the enhancement of the intensity of the 208°C peak and the others would show enhancement of some other peak or the addition of an entirely new peak, should it be different from any component already present in the chromatogram. This was done, with the chromatograms obtained shown on pages 99 , 100, and 101. A chromatogram of the odors trapped from the manure sample used as a blank is given for comparison. It can readily be seen from the chromatograms that the sample which was spiked with the meta isomer of xylene shows the enhancement of the component peak at 208°C, while the chromatograms of the two samples to which the other isomers of xylene were added show enhancement of intensity of signals at other temperatures. This data adds to the validity of the tentative identification of the attractant peak.

The three isomers of xylene were tested as to their attractive capacity for female face flies. The results were inconclusive, the flies being attracted very little to any of the isomers. This result led to the supposition that the attract-

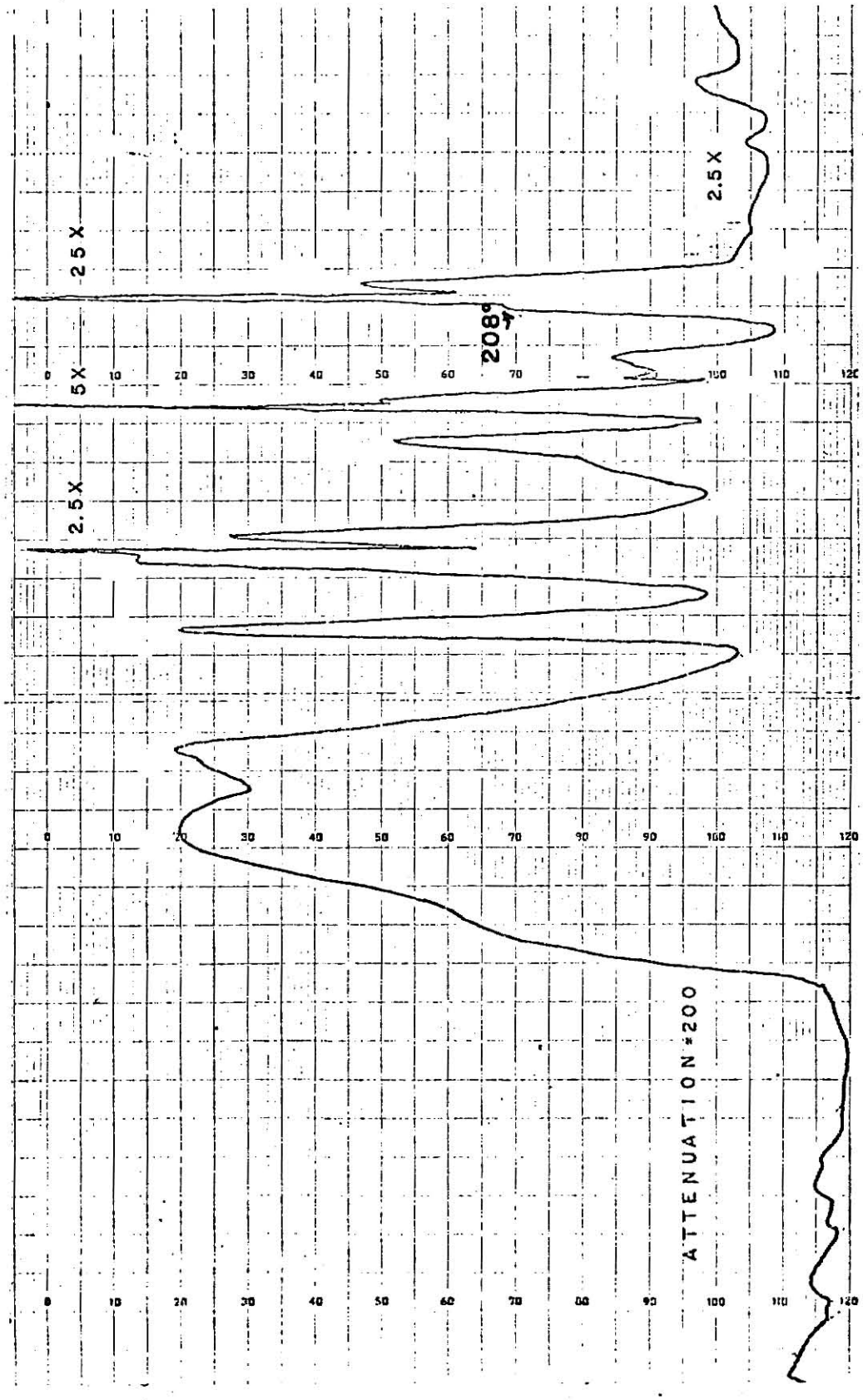
ion of a substance for flies may be concentration dependent. It was then necessary to make up a series of solutions of the meta isomer of xylene, of varying concentrations, and perform the tests again. The NMR spectrometer offers an excellent means of determining the approximate concentration required. This determination was done in the following manner, with the much appreciated assistance of Dr. J. Paukstelis, faculty member of the Chemistry Department, KSU, and an expert in the field of nuclear magnetic resonance spectroscopy.

A stock solution was prepared by adding one microliter of m-xylene to 225 microliters of deuterated chloroform. 33.3 microliters of this stock solution was then added to 300 microliters of deuterated chloroform to give a concentration of 4.93×10^{-1} microliters of m-xylene per milliliter of the deuterated chloroform solvent. An NMR spectrum was taken of this solution. 300 microliters of solvent were then added to the solution in the NMR sample tube, reducing the concentration of the m-xylene to 2.6×10^{-1} microliters of m-xylene per milliliter of solvent. Another NMR spectrum was taken, of this solution. An additional 600 microliters of solvent were added to the sample tube, resulting in a concentration of 1.33×10^{-1} microliters of m-xylene per milliliter of solvent. A third NMR spectrum was obtained, for this sample. From the heights of the peaks on the NMR spectra found at 230 ppm

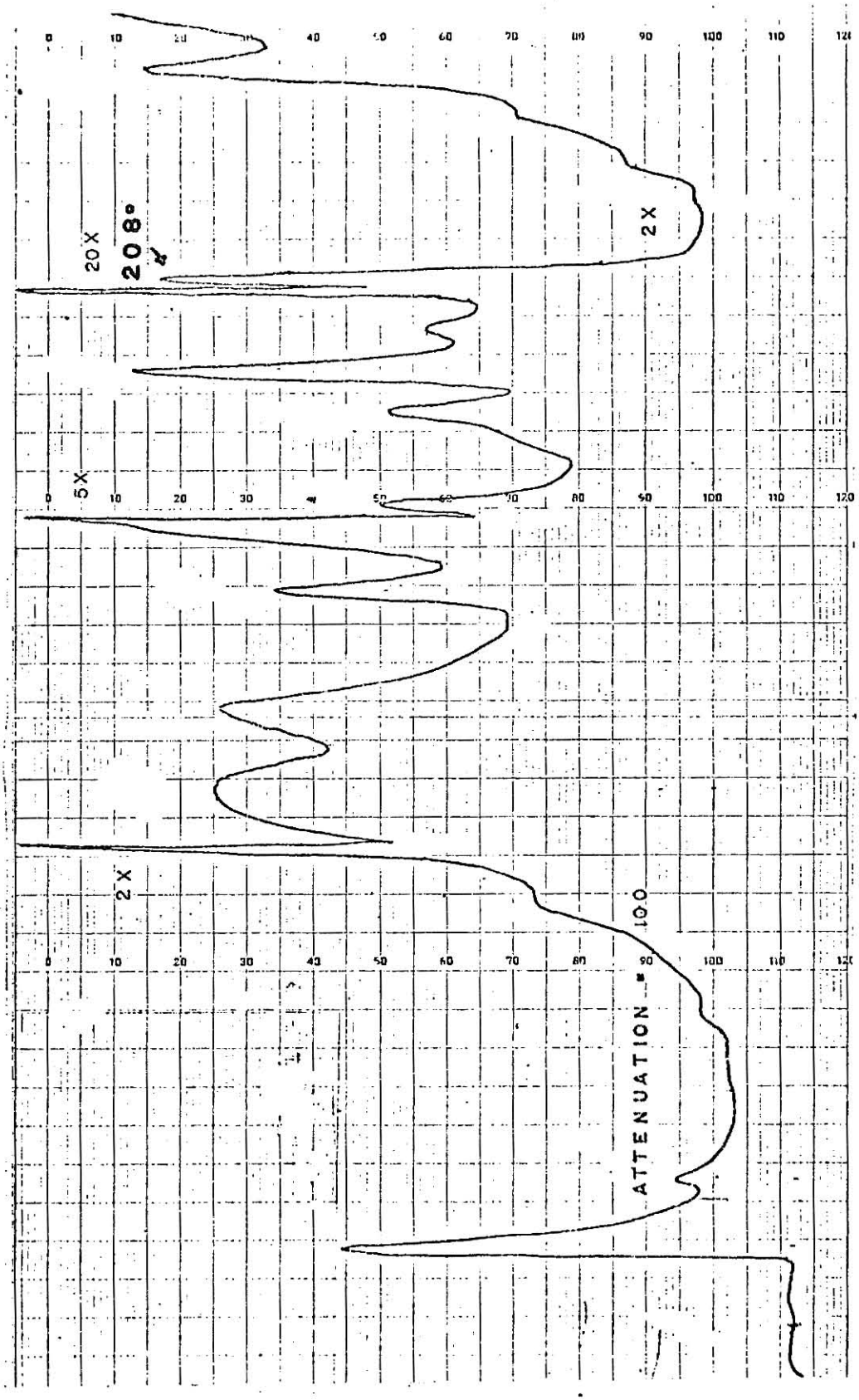
Spectra fifteen; Chromatogram of the odors from a sample of cow manure used as a blank for the xylene spiking experiment.


208°
5X
2X
ATTENUATION=100

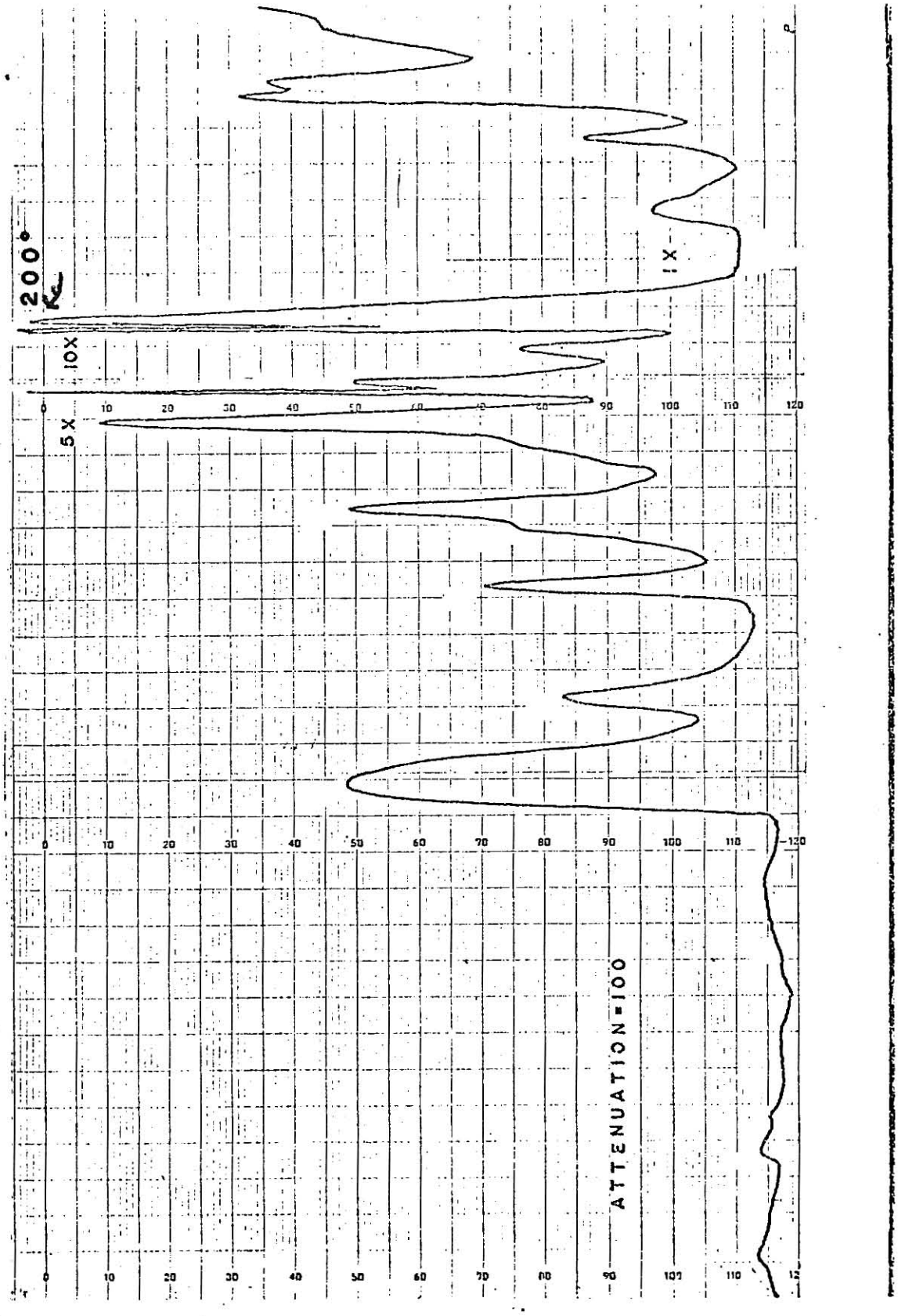
Spectra sixteen; Sample of the odors from a sample of cow manure spiked with the ortho isomer of xylene.


2.5X
2.5X
208°
5X
2.5X
ATTENUATION=200

Spectra seventeen; Chromatogram of the odors trapped from a sample of cow manure spiked with the meta isomer of xylene.


20X
208°
2X
5X
2X
ATTENUATION 10.0

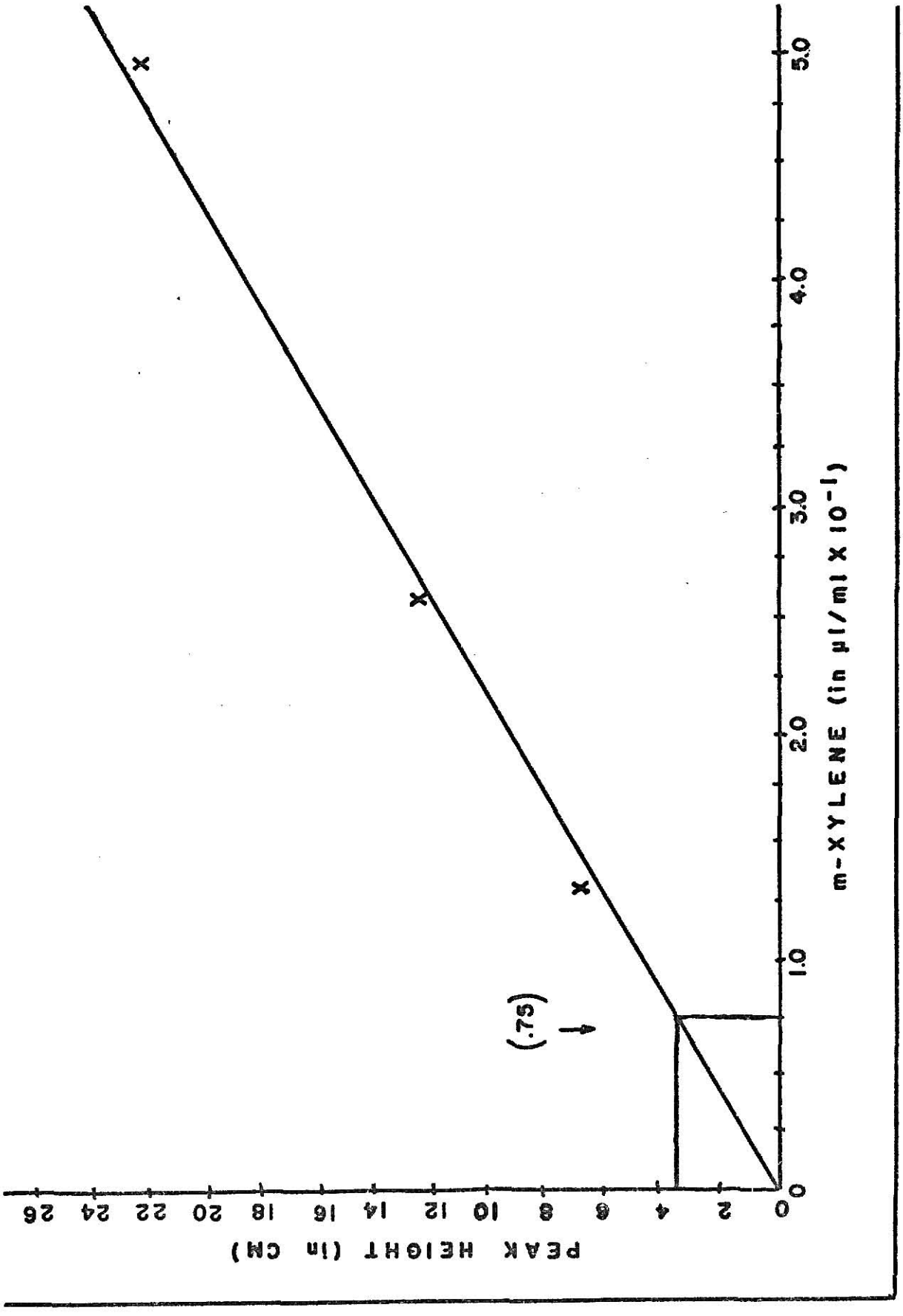
Spectra eighteen; Chromatogram of the odors trapped from a sample of cow manure spiked with the para isomer of xylene.


200°
1X
10X
5X
0
10
20
30
40
50
60
70
80
90
100
110
120
ATTENUATION=100

and the concentration of the solutions which produced these signals, a calibration curve was drawn. Using this curve, and the height of the appropriate peak on the NMR spectrum obtained from the 208°C peak from the manure sample, the approximate concentration of the reactant component in the 13 peaks collected from the gas chromatographic column was found to be 0.75×10^{-1} microliters per milliliter of solvent. For a single, eluted component from the gas chromatographic column, this would correspond to a concentration of approximately 5 parts per million.

Solutions of m-xylene in water were prepared for a test with female face flies in the following manner. Commercial xylene is soluble in water to the extent of 0.014 g/100 ml. The specific gravity of m-xylene is 0.86417 g/ml at 20°C. (Encyclopedia of Technology). This corresponds to a concentration of 160 parts per million. A solution of 160 parts per million m-xylene in water was prepared and dilutions made to give solutions of 0.4, 0.8, 1.60, 8.0, and 16.0 parts per million m-xylene in water. The 208°C peak eluting from the gas chromatographic column was trapped from a fresh manure sample and the odor from the 'paunch pellets' was trapped in a small quantity of Tenax. Stock solutions of para-xylene and ethyl benzene were also placed on small portions of Tenax. All of these samples were then tested with female face

Figure eighteen; plot of calibration curve from NMR spectra for determination of the approximate concentration of the attractant peak eluted from the gas chromatographic column at 208^{o}C.


PEAK HEIGHT (in CM)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
m-XYLENE (in µl/ml X 10⁻¹)
0
1.0
2.0
3.0
4.0
5.0
(.75)

flies which were ready for egg-laying. The results of these tests are given in the table below.

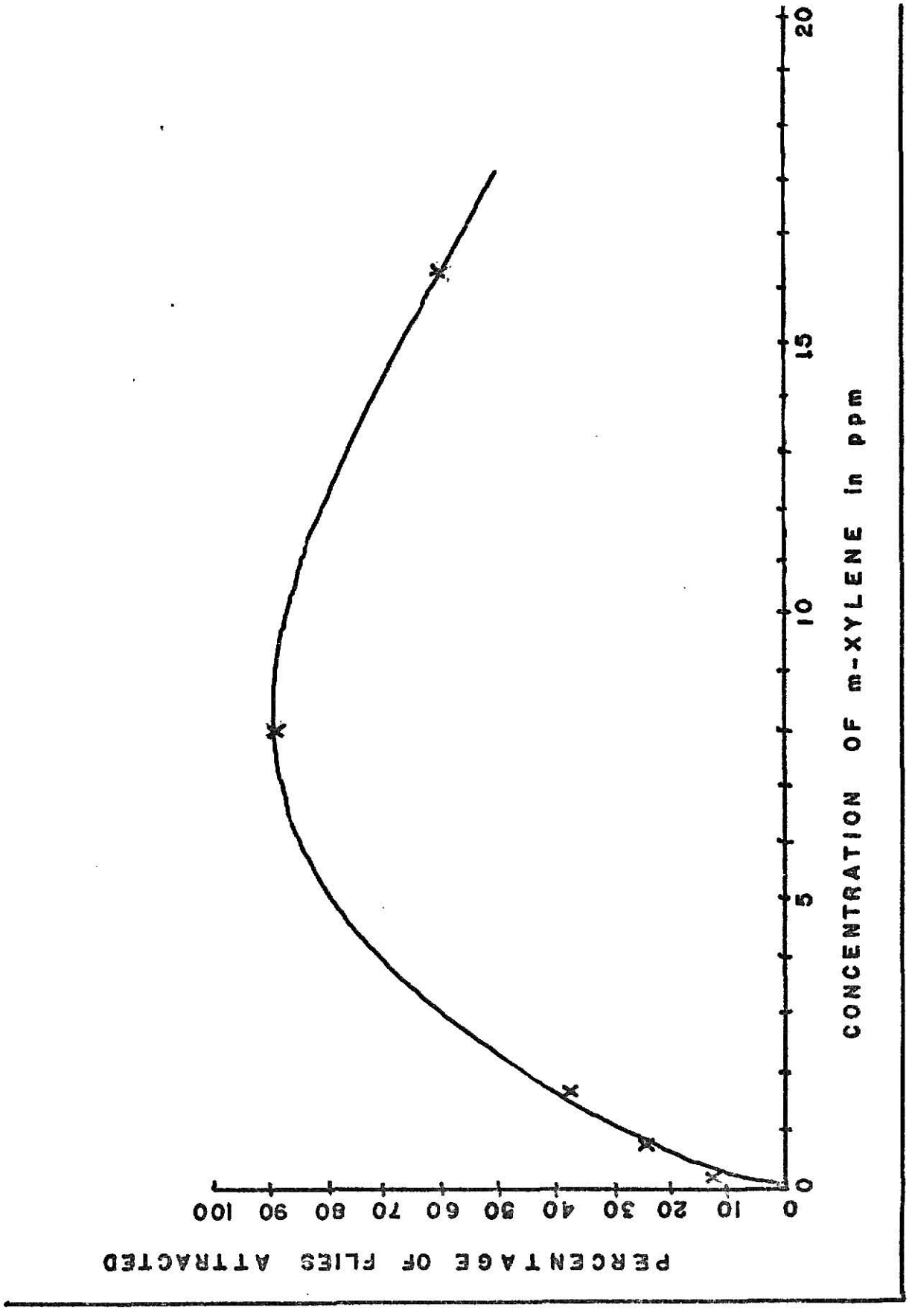
Sample	Total Flies	Sample Side	Blank Side	Unreacted
208°C peak	32	30	1	1
Paunch pellets	28	19	4	5
p-xylene	28	11	5	12
Et-benzene	26	7	4	15
m-xylene solutions				
0.4 ppm	16	2	0	14
0.8 ppm	20	7	1	12
1.6 ppm	24	9	1	14
8.0 ppm	28	25	0	3
16.0 ppm	25	15	2	8

All tests were performed for a period of five minutes.

This was the best response exhibited by the flies to the trapped odors thus far. All of the aqueous solutions were placed on small quantities of Tenax and placed in the side-arm flask of the olfactometer, exactly as previous samples had been tested. The results of the test with these solutions were graphed and are shown on page 106.

Fresh solutions of m-xylene in water were prepared and the tests conducted on a new group of female face flies. The results of the test are shown in the table, page 107 and the

Figure nineteen; plot of female face fly response versus concentration of meta xylene in water solution.


PERCENTAGE OF FLIES ATTRACTED
0
10
20
30
40
50
60
70
80
90
100
CONCENTRATION OF m-XYLENE in ppm
0
5
10
15
20

graph of the response is found on page 108 . The results are very nearly the same as in the previous test. It may be that the actual optimum concentration of the m-xylene for attraction of flies is slightly in error, due to the extremely small concentrations of the solutions used and the possibility of an error being made in preparation of solutions with such low solubility and extremely small quantities of solute being used.

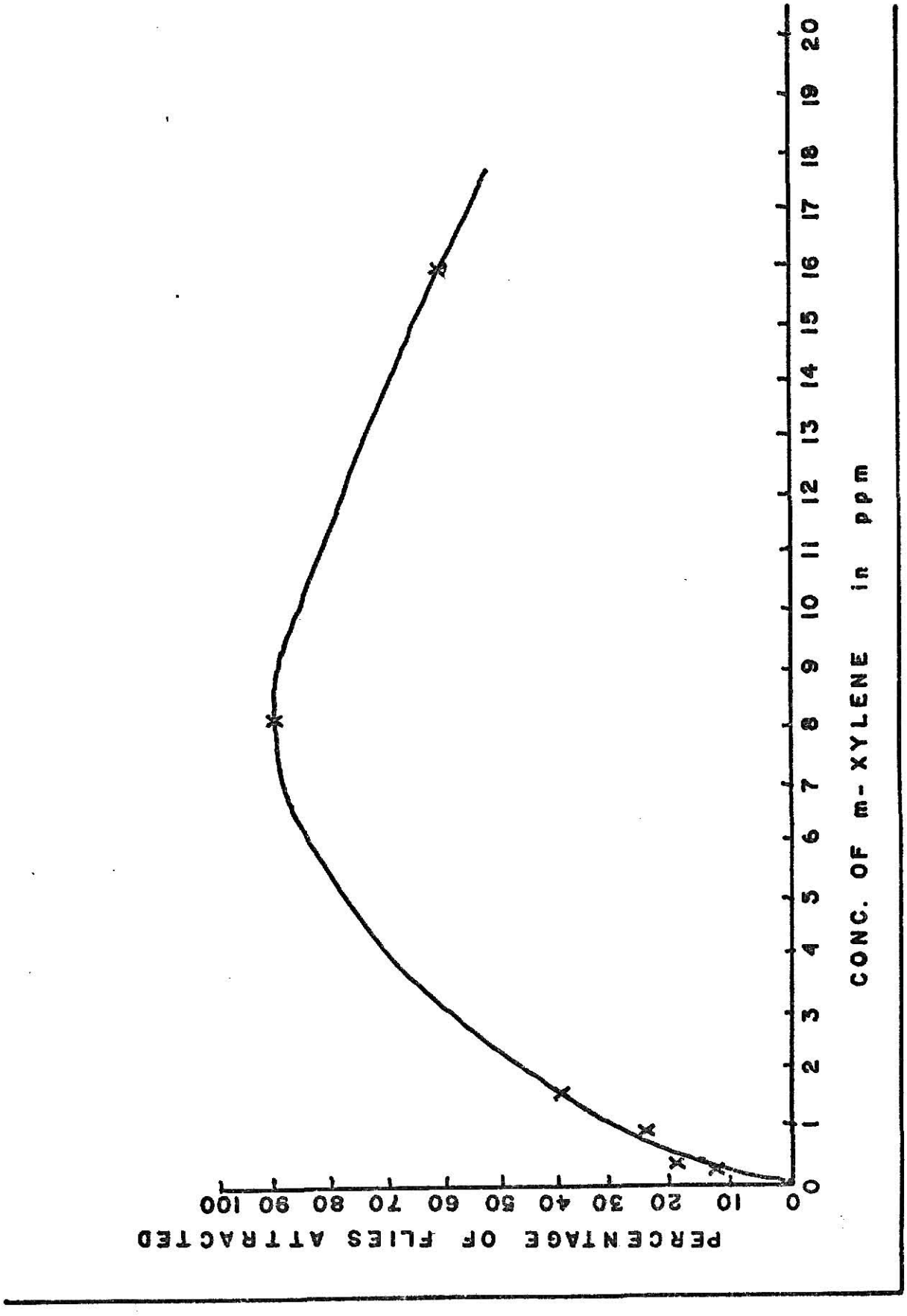
The testing of the solutions of para-xylene and ethyl benzene were not repeated, since the mass spectral data and the NMR data did not conform to the literature values for these compounds. It is possible, however, that these compounds may have a greater attraction for female face flies than observed in the test discussed earlier, if present in the proper concentration. They do not seem to be the component of interest in the cow manure samples being studied at present.

Sample	Total Flies	Sample Side	Blank Side	Unreacted
0.16 ppm	21	3	1	17
0.40 "	20	4	0	17
0.80 "	24	6	2	16
1.60 "	18	7	1	10
8.00 "	29	26	2	1
16.0 "	31	19	3	9

All samples were tested for five minutes. Graph on page 108.

Determination of the attractive component of the cow manure as m-xylene raised some doubt as to the feasability of its

Figure twenty; plot of female face fly response versus concentration of meta xylene in water solution.


PERCENTAGE OF FLIES ATTRACTED
0
10
20
30
40
50
60
70
80
90
100
CONC. OF m-XYLENE in ppm
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

identity. Since the compound has been known for such a very long time, and has never been observed to be a fly attractant, possible sources of xylene contamination were investigated. It is thought that the reason for its lack of attraction for flies in work making use of this compound is perhaps due to its concentration dependency. The tubing used in the collection of volatile materials from the manure samples was made of Tygon. Since many plastics have trace amounts of xylenes in their composition, this was the most likely source of contamination. In order to determine whether this was the case, the following experiment was performed. With no samples in the blenders, the trapping system was operated in the same manner as if samples were present. The Tenax-GC used in trapping was treated in an identical manner as if it indeed did contain trapped volatiles from a manure sample. Upon being chromatographed, there was found to be only a steady baseline and no peaks. This was taken as excellent evidence that the meta-xylene came from the manure samples and not from some source of contamination. The same plastic tubing was used to make connections in the trapping system throughout the entire project.

Another source of possible contamination might have been in the carrier gas or tubing system conducting the carrier to the gas chromatographic injection system. This was checked

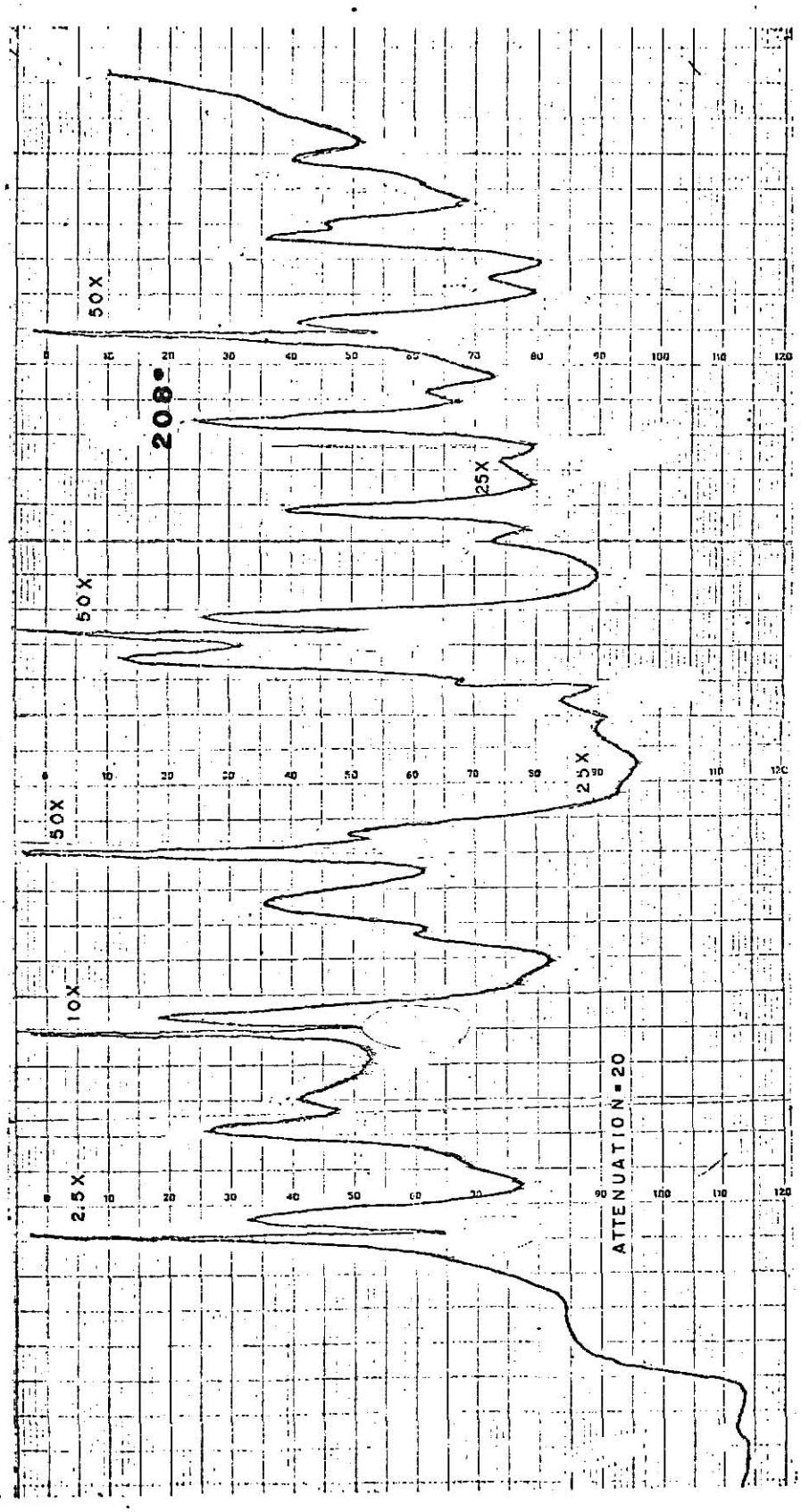
by two methods. A sample of Tenax containing no sample for manure odors was used in an identical manner as if it did contain a trapped volatile portion from a manure sample. When chromatographed, no peaks were evident. The column effluent was then trapped and introduced into the mass spectrometer. When this was done, no relative ion current was seen, other than a small group of peaks due to a small amount of air being introduced into the sample chamber of the mass spectrometer along with the sample.

The two experiments just described were taken as evidence that the meta-xylene identified as the attractive component for the flies in the manure did indeed come from the manure samples and not from some source of contamination during the experiments performed.

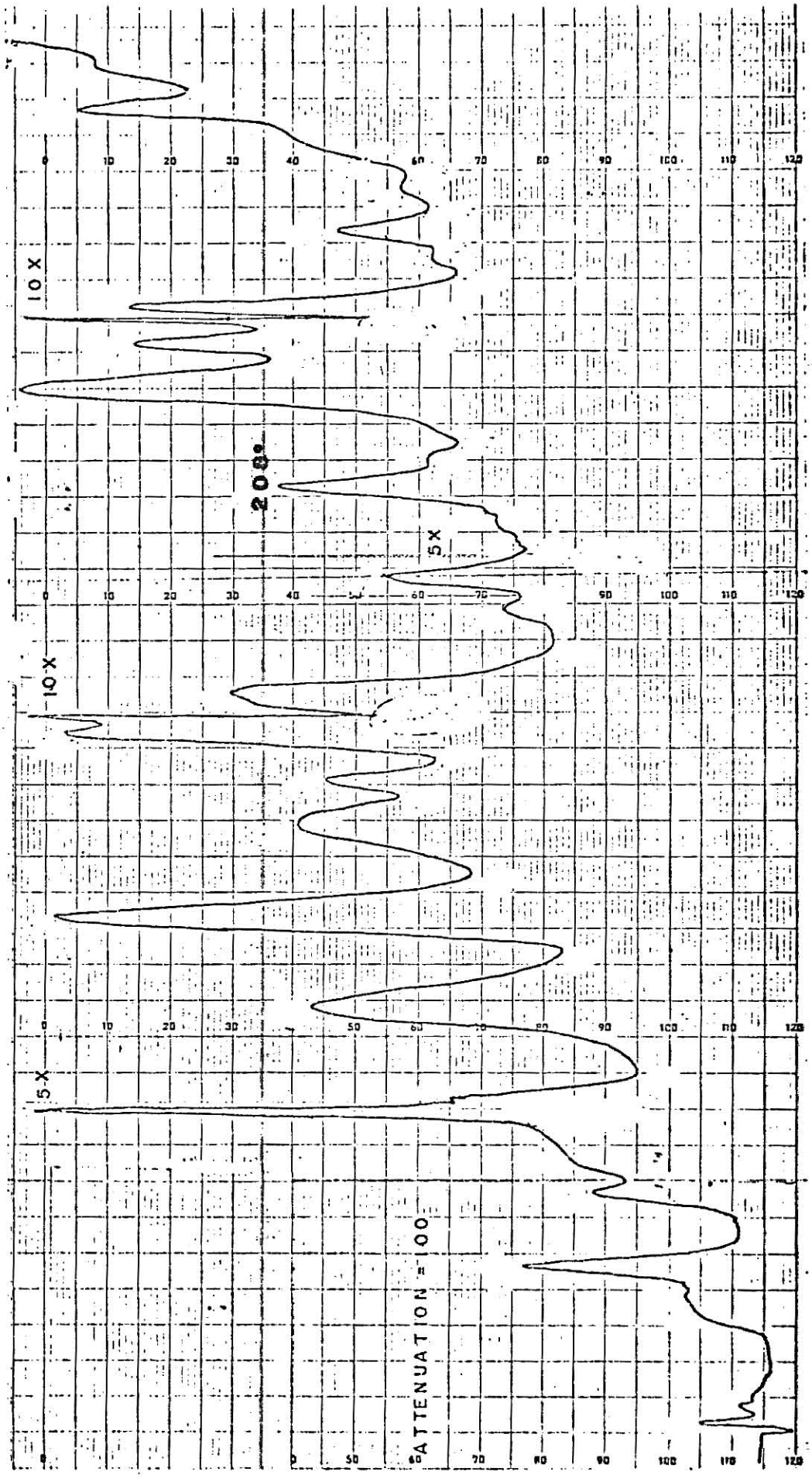
Samples of manure from other animals and chickens were collected to determine whether or not the peak which elutes from the gas chromatographic column at 208°C was present. Since it is known that manure from these sources will also attract flies, (Scott and Littig, 1960), it was desired to determine if the same component may be responsible for this attraction. Samples of manure from a horse, sheep, goat, hog, dog and chickens were collected, the volatile odors trapped as before, and chromatographed. The chromatograms obtained are shown on

the following pages. Only the horse and sheep reactant peaks were trapped as they eluted from the column and tested on female face flies. The attraction was the same as noted for the reactant peak trapped from cow manure. It can be seen from the gas chromatograms, that the eluted 208°C peak is present in all manure samples except those collected from a dog and a hog. In conferring with the owner of the dog, it was found that the animal was maintained on a diet consisting primarily of meat. This may explain the absence of the peak, since it has only been found to be present in samples from animals which are fed material of plant origin, particularly plants high in cellulose content. The chromatogram of the odors from hog manure does not show the reactant peak, but this may be due to its being masked by some other constituent. The chromatogram is not a very good one, some instrumental problems being dealt with at the time it was taken. The gas chromatogram of the volatile compounds contained in goat manure shows the peak of interest to be quite small. Checking with the owner of the goat produced the information that the goat was of the milking variety and is maintained on a diet consisting of a high percentage of grain. This may account for the small proportion of the reactant peak. The chicken manure sample was taken from the bottom of a cage which contained four days accumulation of droppings. The reactant peak at 208°C is seen to be present in very high concentration

Spectra nineteen; Chromatogram of the odors trapped from a sample of horse manure.

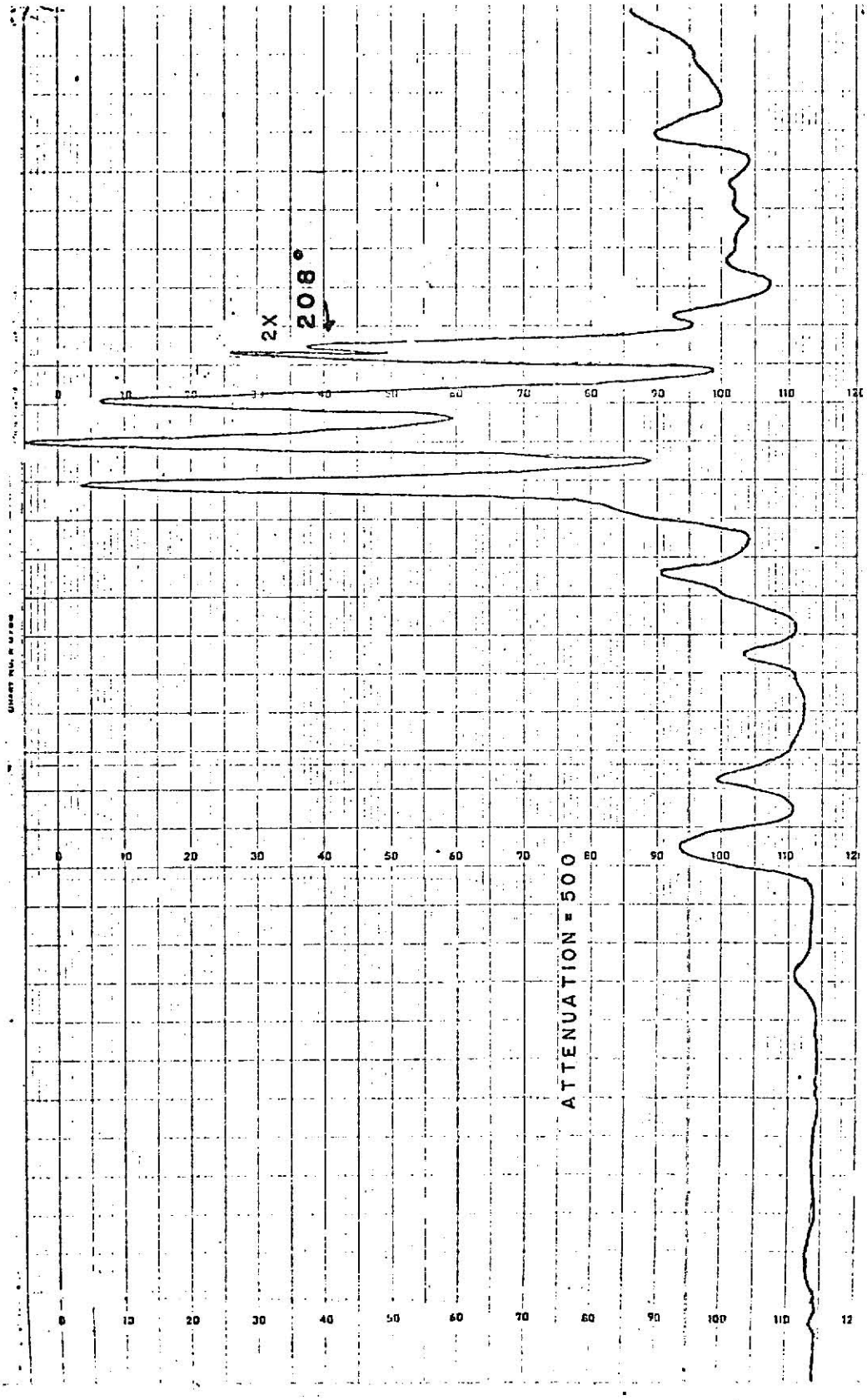

208°
50X
25X
50X
25X
50X
10X
2.5X
ATTENUATION = 20

Spectra twenty; Chromatogram of the odors trapped from a sample of sheep manure.

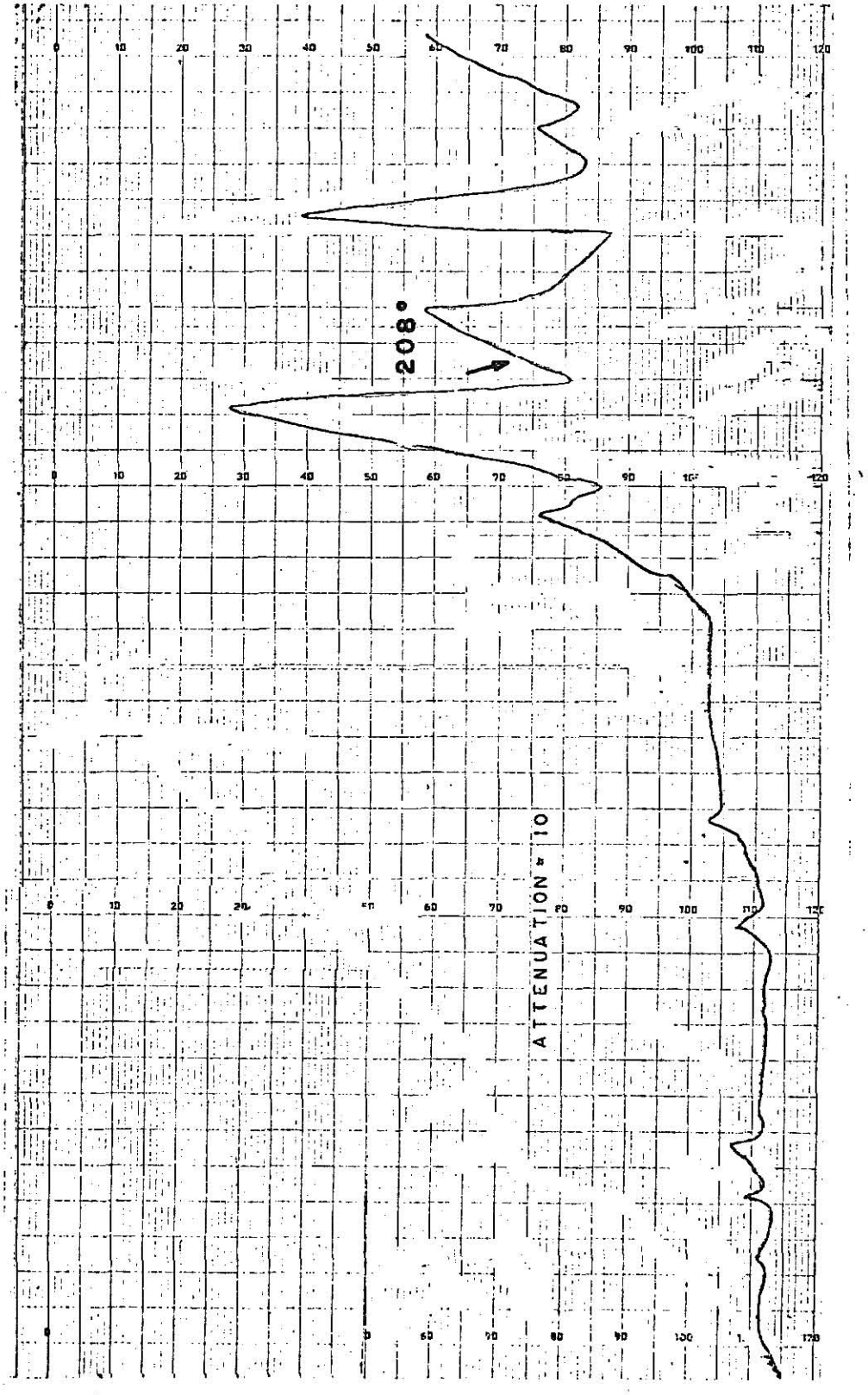

10 X
200°
5X
10X
5X
ATTENUATION = 100

Spectra twenty-one; Chromatogram of the odors trapped from a sample of chicken manure

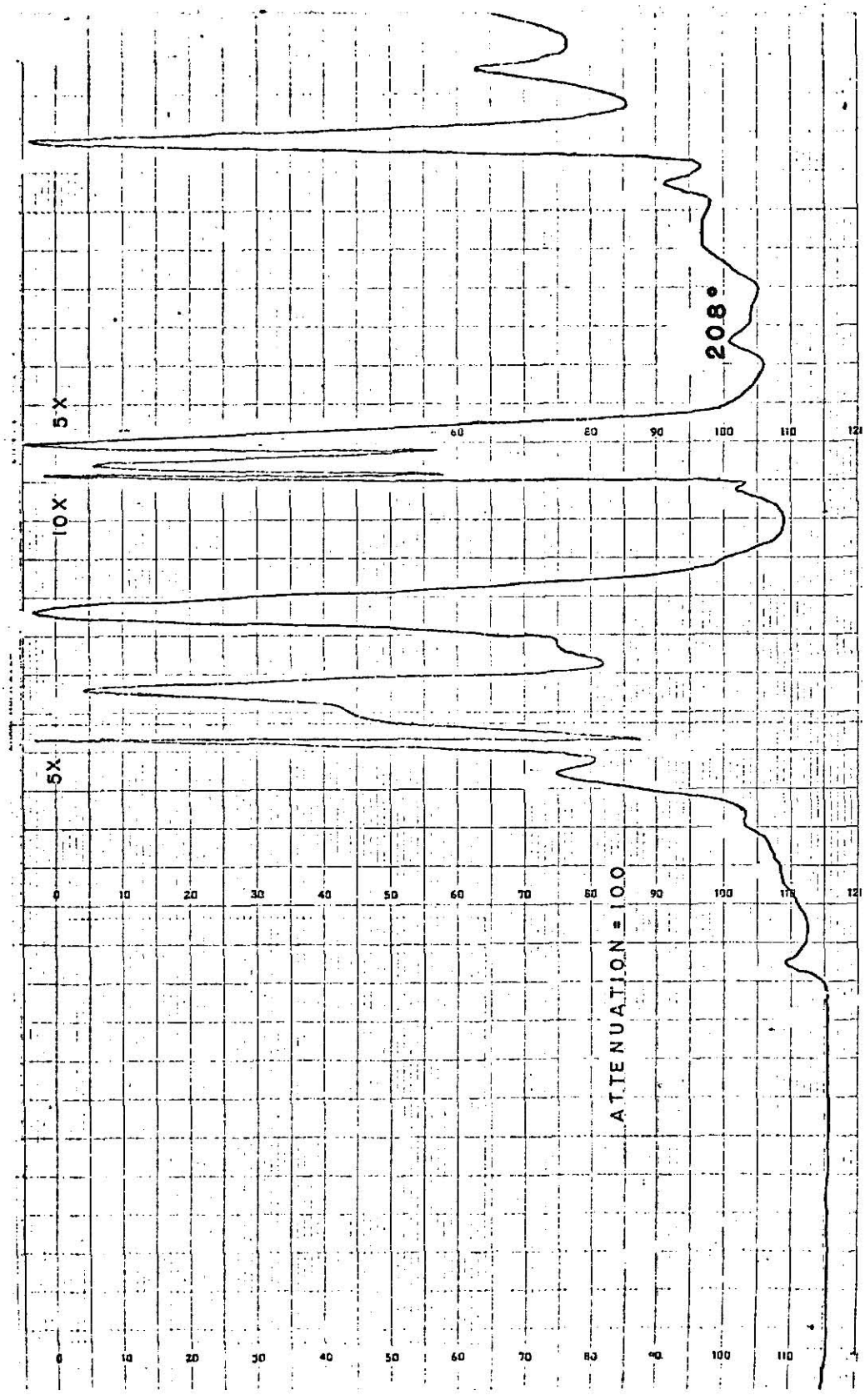
(white leghorn variety)


2X 20.8°
ATTENUATION = 500

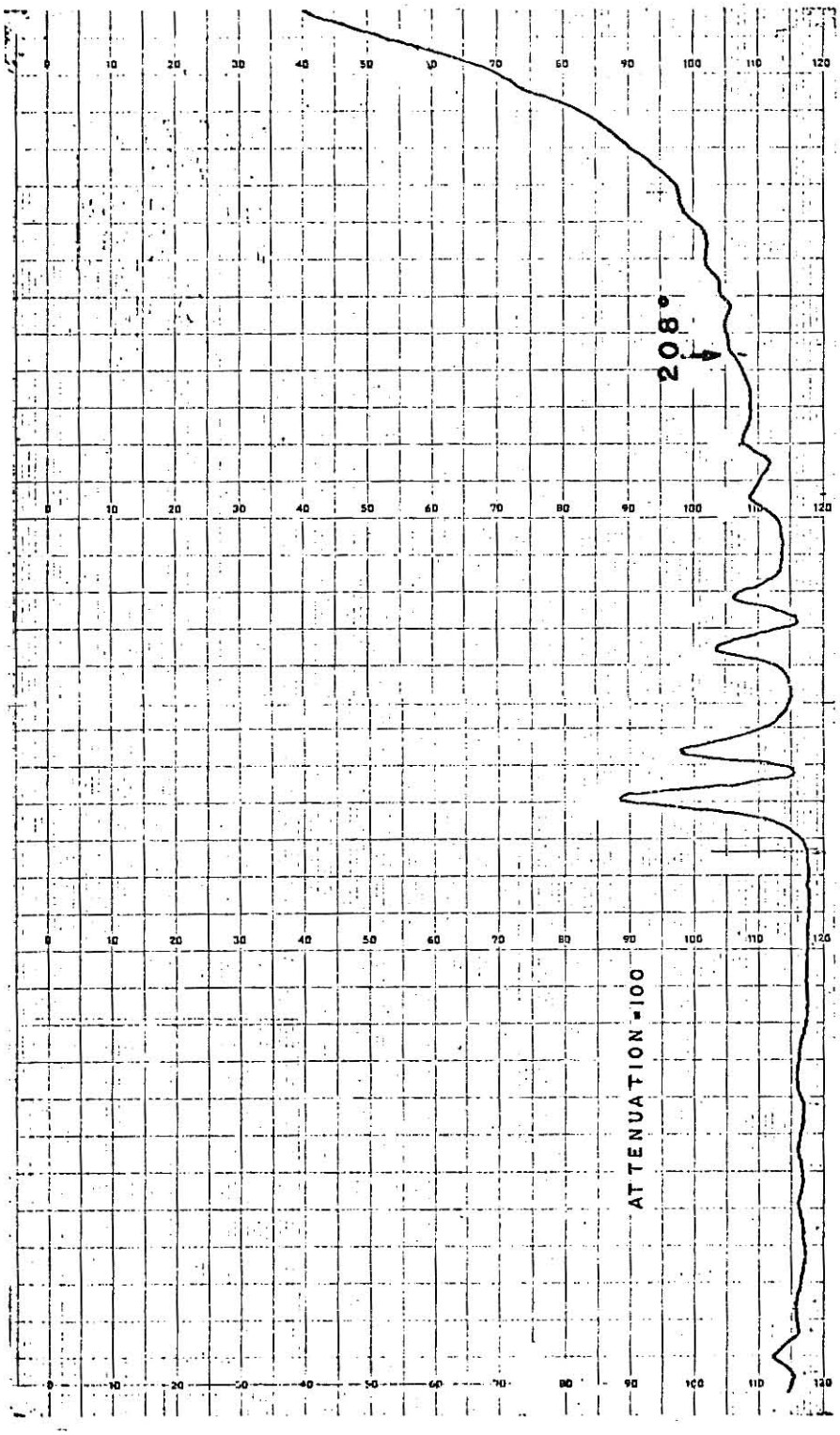
Spectra twenty-two; Chromatogram of the odors trapped from a sample of Duroc Hog manure


208°
ATTENUATION = 10

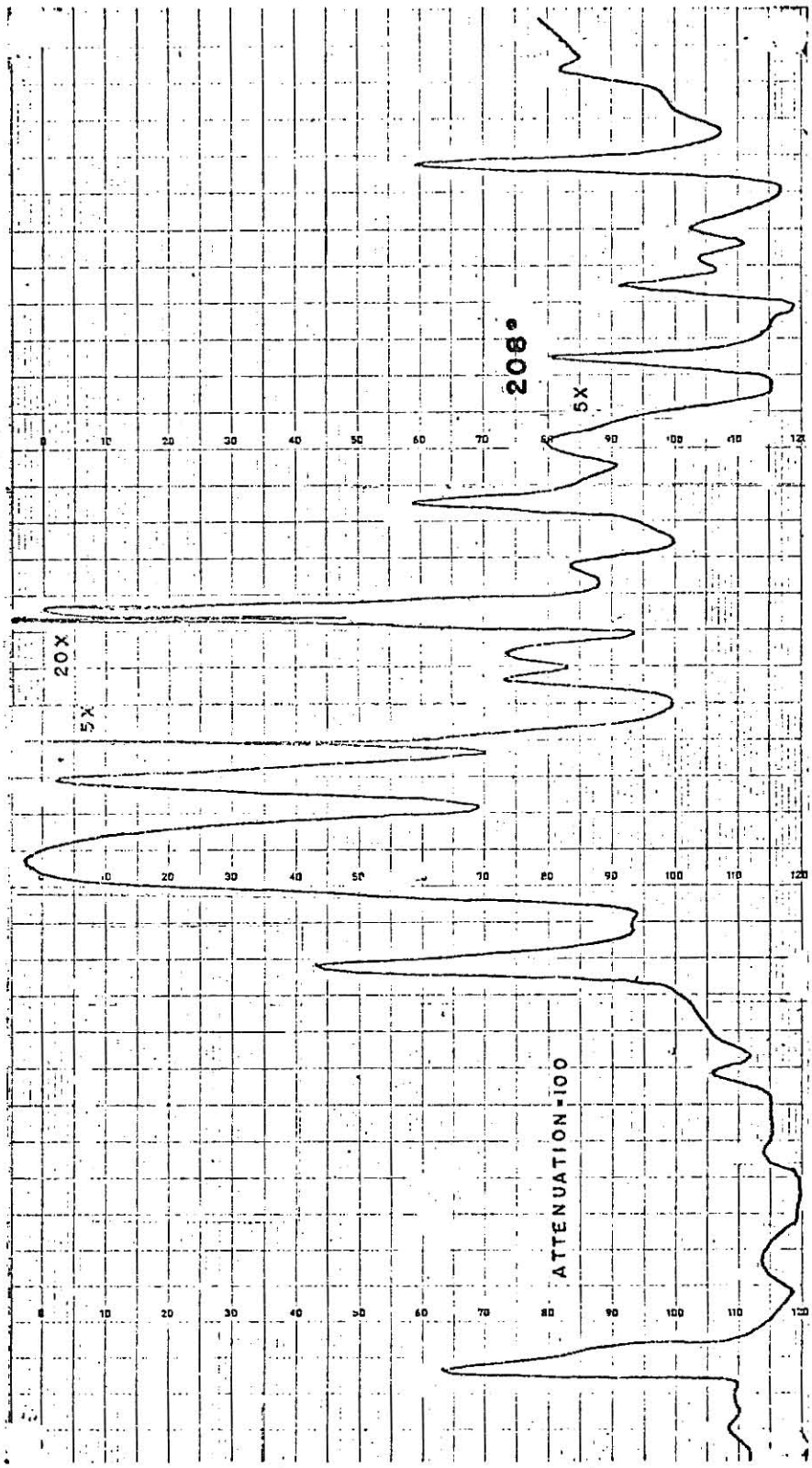
Spectra twenty-four; Chromatogram of the odors trapped from a sample of goat manure.


208°
5X
10X
5X
ATTENUATION = 10.0
0
10
20
30
40
50
60
70
80
90
100
110

Spectra twenty-five; Chromatogram of odors trapped from a sample of dog manure.


208°
ATTENUATION=100

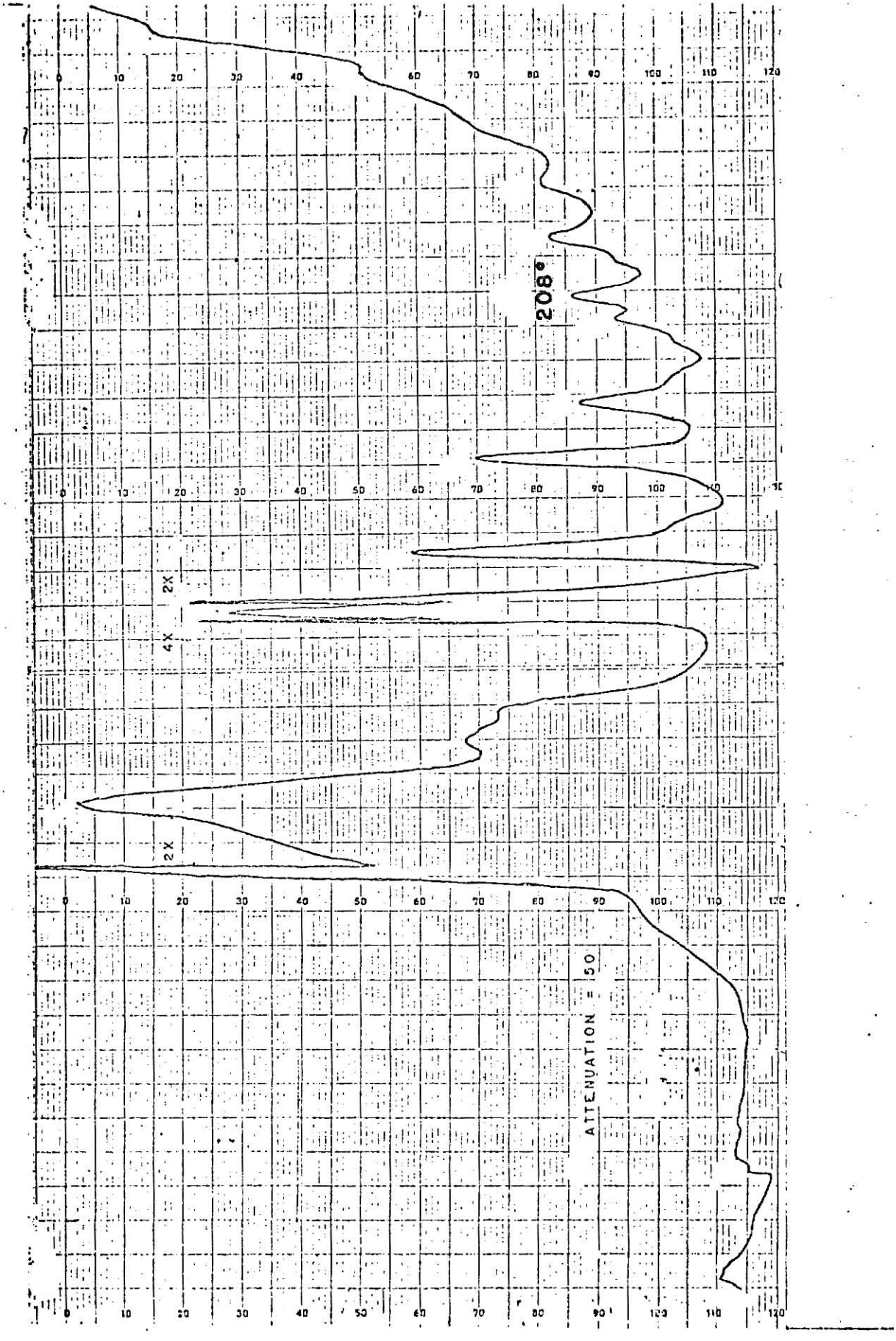
Spectra twenty-six; Chromatogram of odors trapped from a sample of 'paunch pellets.'


208°
5X
20X
5X
ATTENUATION=100

and this is in accord with the information presented in the introduction.

Garbage is almost always the most important source of house flies in urban areas, (Scott and LIttig, 1960). This fact prompted the investigation of garbage odors to determine if the same component is present in garbage as in the animal sources which are known to be common breeding media for the face flies on which the tests have been conducted. Food scraps were placed in the blenders used in the odor-trapping apparatus and the odors trapped on Tenax as for the manure samples. The volatile materials responsible for the odors were then chromatographed and the resulting chromatogram is shown on the following page. The food scraps were then allowed to ferment at room temperature for two days and the sampling of the odors repeated as before. It can be seen from the chromatogram on page 122 that the eluted 208°C peak which was present in the chromatogram of the food scrap odors has become more intense, relative to the other peaks. This garbage sample was allowed to ferment for an additional two days and the sampling process, with subsequent chromatographing of the odors, resulted in still greater enhancement of the peak of interest. This chromatogram is found on page 123 . This is interpreted as the odor which is responsible for the attraction of the flies being a bacteriological metabolite. By

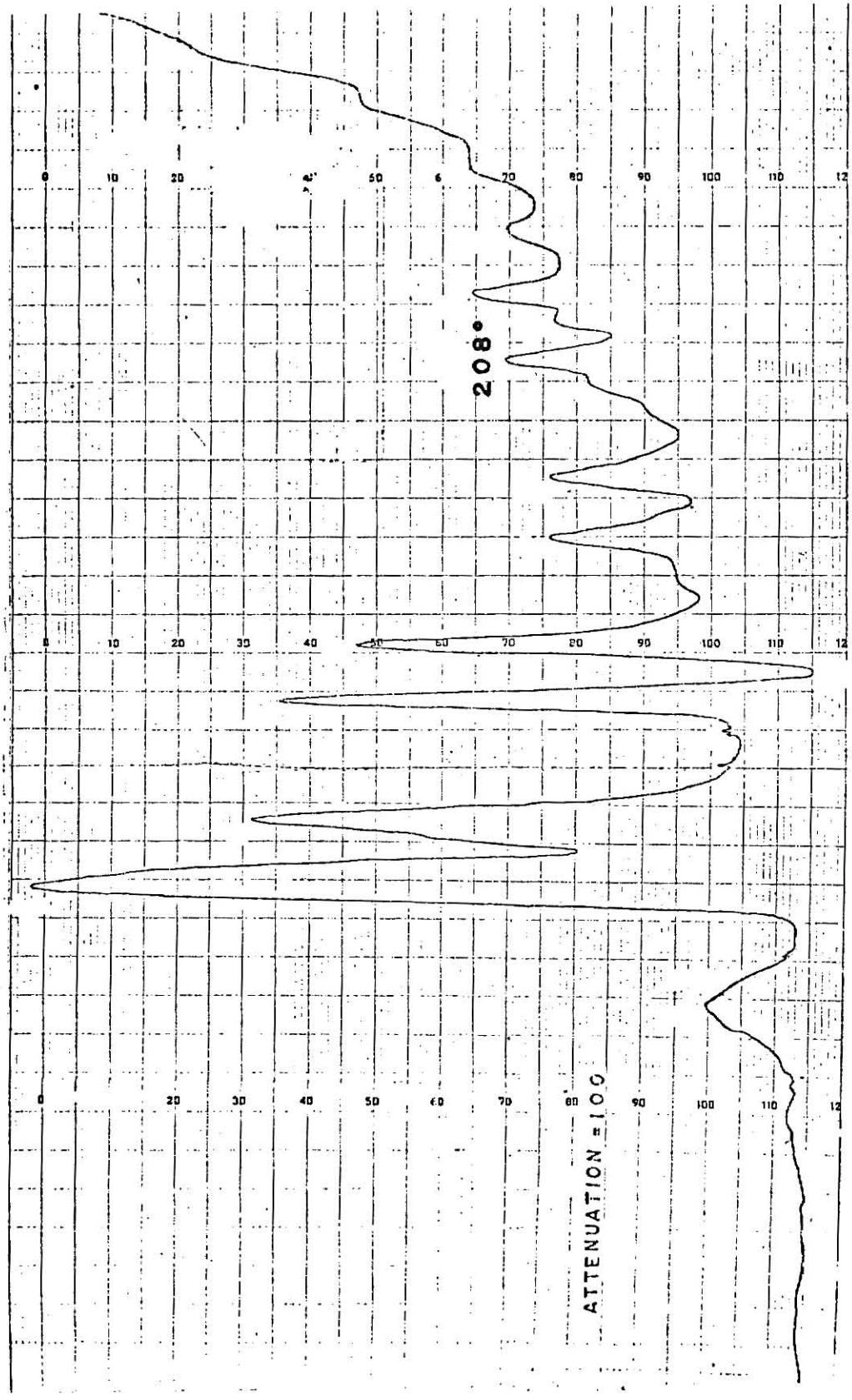
Spectra twenty-seven; Chromatogram of the odors trapped from a sample of fresh food scraps.


208°
2X
4X
2X
ATTENUATION = 50

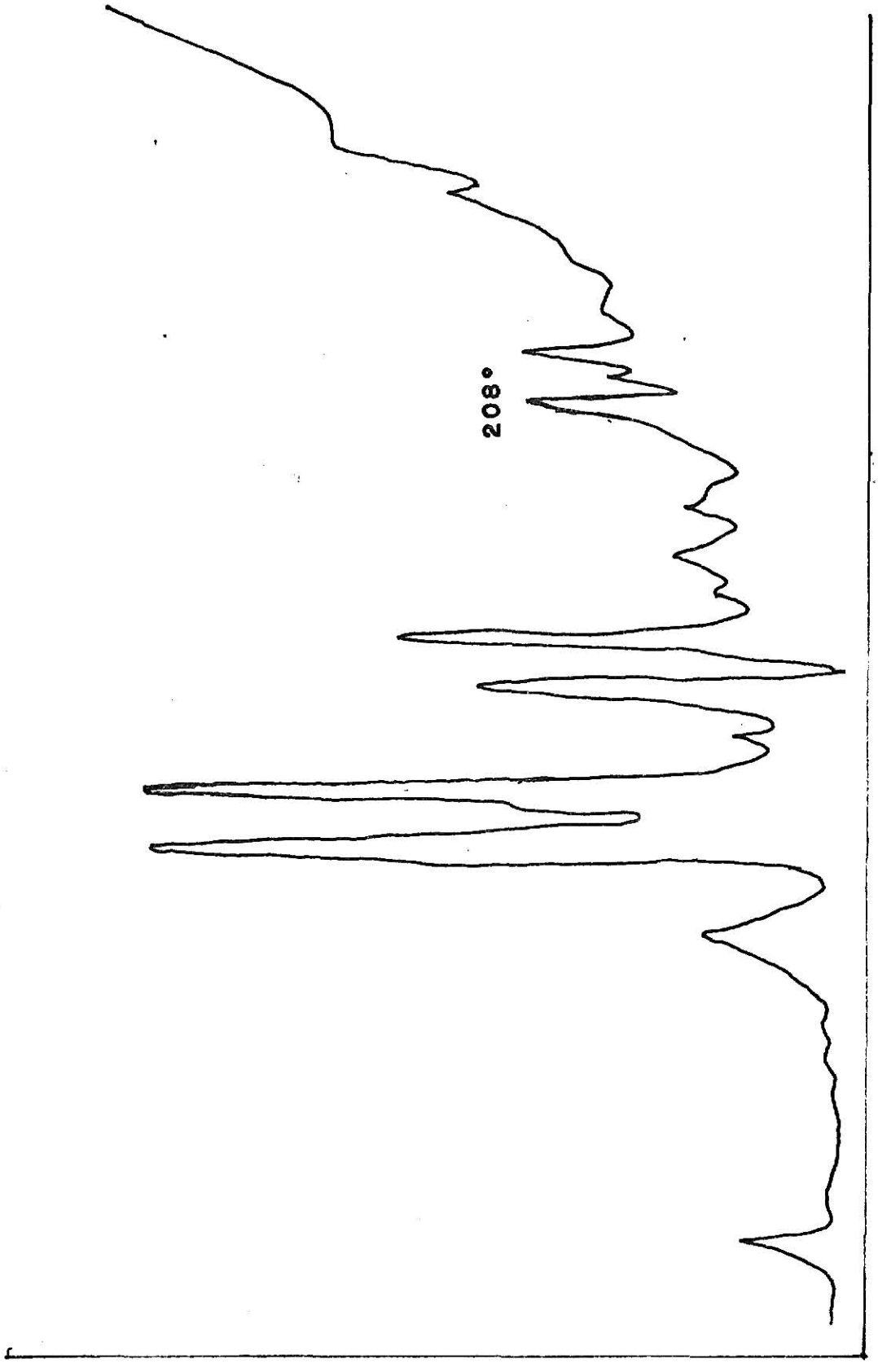
extrapolation, this may also be a true indication of the digestive process taking place within the animal, since the attractive material appears to be the same in both cases. The garbage sample was allowed to age undisturbed for a period of two weeks and the odor sampling process repeated. The 208°C peak had now diminished to the point where it was no longer significant. The volatile component responsible for its existance has now evaporated and is no longer capable of attracting the flies. This is in accord with the observable rate of decline of fly populations in and around garbage which has become dried out through exposure to air and sunlight.

Extension of this work to other substances which are known to attract flies in abnormal concentrations would be interesting and perhaps most informative. Time did not allow this extension of this work, with the exception of one other material, that being the ribbon-type fly-catcher paper used in many homes in earlier years, and still in use today. One of these ribbon fly-catchers was placed in a blender and the sampling-chromatographing process carried out. The gas chromatogram presented on page 125 shows the presence of the component peak at 208°C. The manufacturer does not list this component as one of the active ingredients in the composition of the product.

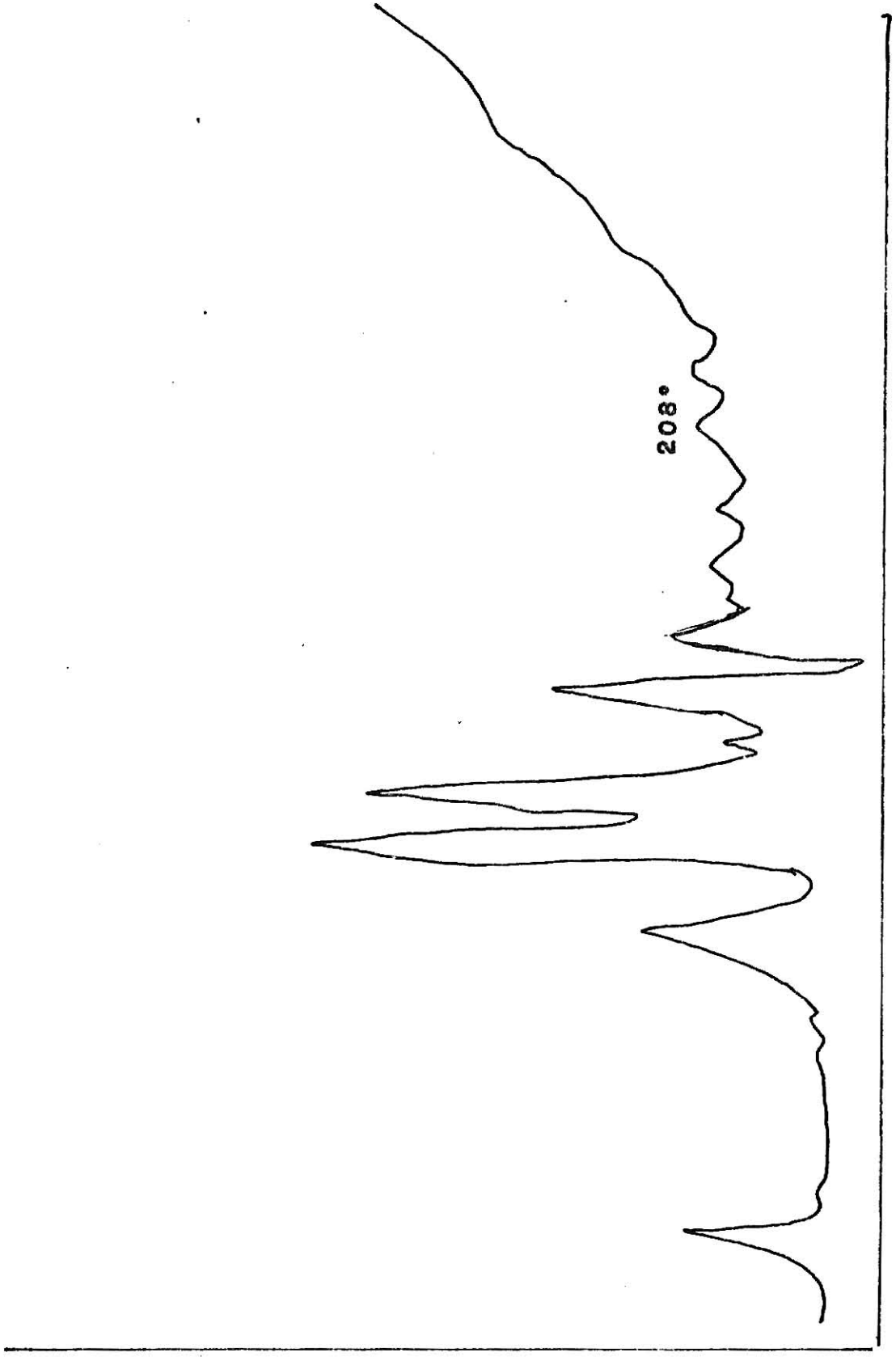
Spectra twenty-eight; Chromatogram of odors trapped from a sample of garbage after aging for a period of two days.


208°
ATTENUATION = 10 C

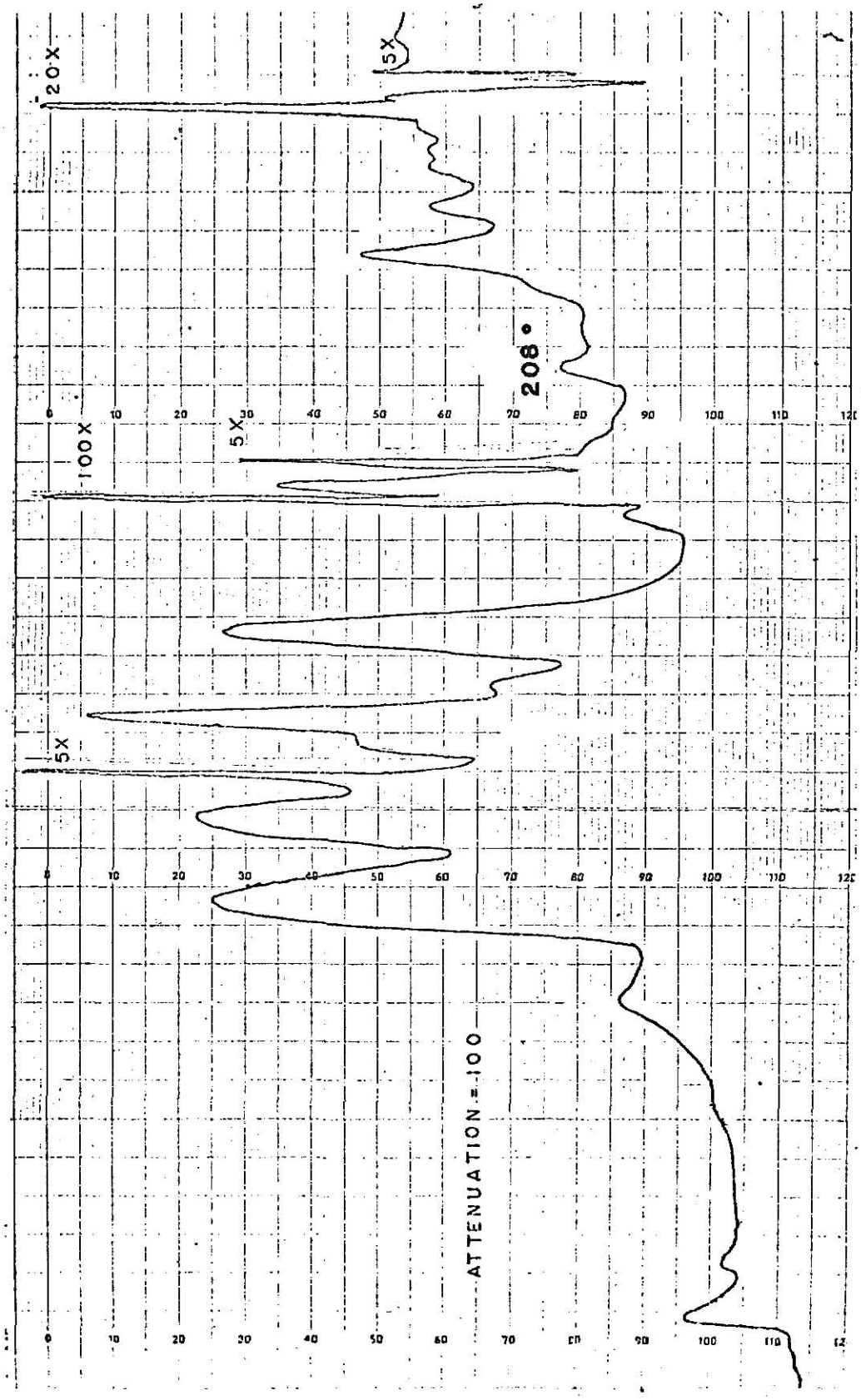
Spectra twenty-nine; Chromatogram of odors trapped from a sample of garbage allowed to age for a period of four days.


208°

Spectra thirty; Chromatogram of odors trapped from a sample of garbage two weeks old.


208°

Spectra thirty-one; Chromatogram of odors trapped from a sample of ribbon-type fly-catcher paper.

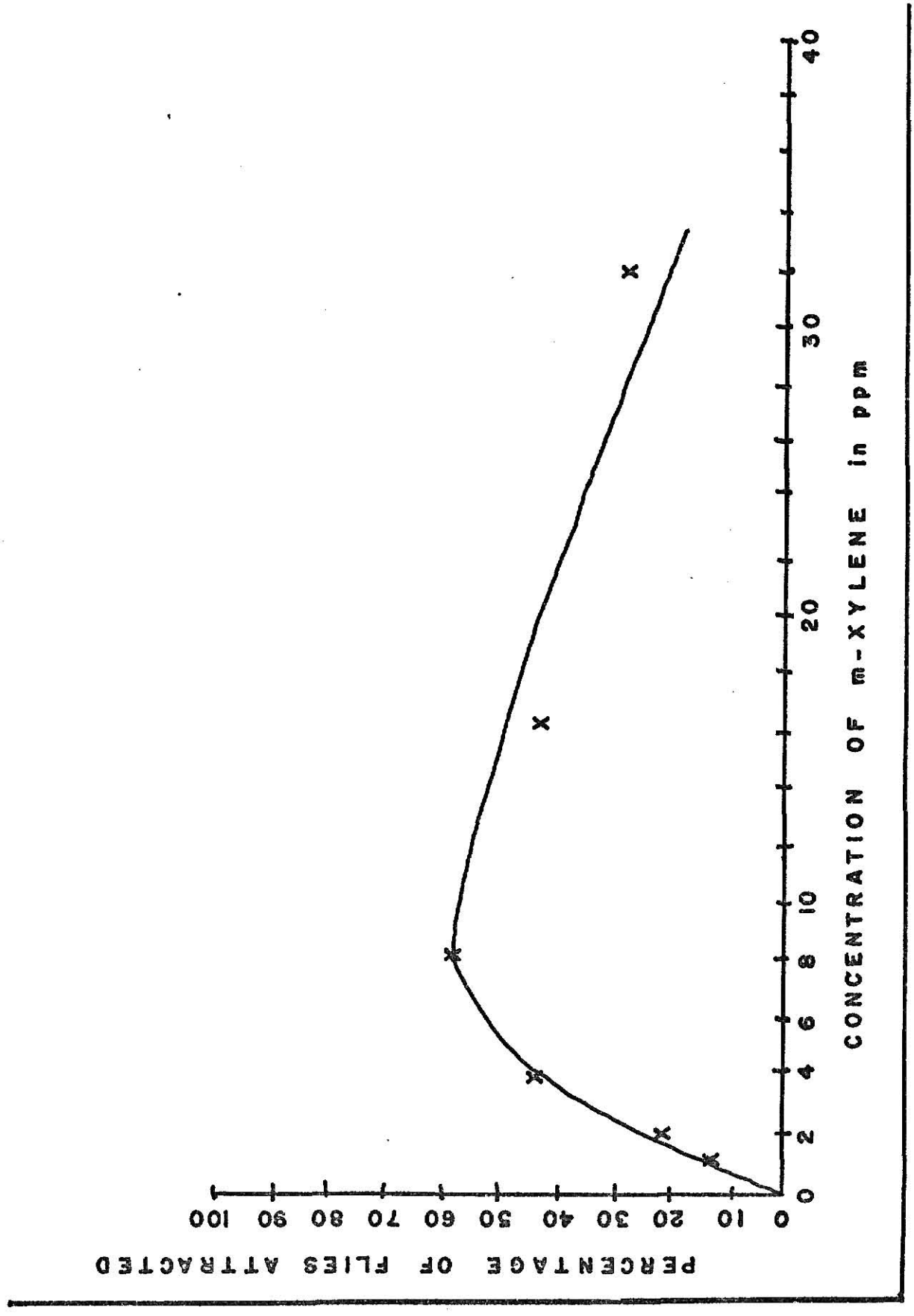

20X
5X
208°
100X
5X
5X
ATTENUATION = 100
0
10
20
30
40
50
60
70
80
90
100
110

The final experiment to be carried out in this work was the testing of gravid female houseflies with respect to the attraction of aqueous solutions of m-xylene. The solutions were prepared as before, in slightly different concentrations, and the flies tested through the use of the olfactometer. When the experiment was ready to be performed, it was found that the houseflies had not been sexed. The data presented in the table below would be in even better agreement with the data obtained on female face flies, had the separation of the female and male flies been done. A plot of the fly response is found on the following page.

Sample	Total Flies	Sample Side	Blank Side	Unreacted
1.00 ppm	18	2	1	15
2.03 "	22	4	0	18
4.05 "	25	11	1	13
8.10 "	34	19	5	10
16.2 "	32	14	6	12
32.4 "	29	10	5	14

All tests were performed for a period of five minutes.

Figure twenty-one; plot of house fly response versus concentration of meta xylene in water solution (these flies were not sexed).


PERCENTAGE OF FLIES ATTRACTED
0
10
20
30
40
50
60
70
80
90
100
CONCENTRATION OF m-XYLENE in ppm
0
2
4
6
8
10
20
30
40

Fly Information

Dr. C. Pitts, KSU Entomologist, has investigated the external anatomy of the face fly through the medium of the electron microscope. The female face fly, in determining the loacation of egg deposition, probes the surface of the material with her antennae and ovipositor, (Bay, 1974). The anal leaflets of the ovipositor have been concluded to be olfactory by other workers, (Wallis, 1962). Female face flies have been observed, in the experiments conducted in this work, lowering their ovipositors in fresh manure samples, in Tenax-GC containing the trapped attractive component, and in scattered particles of the Tenax containing the trapped attractive component, inadvertently spilled on the surface of the table during the olfactometer tests. It seems reasonable to conclude that flies are not able to, or do not discriminate between, the actual manure samples or the Tenax containing the trapped attractive component from the manure sample.

Conclusion

The data presented in the tables, chromatograms and graphs on the pages earlier, are taken as excellent evidence for the isolation and identification of the natural fly attractant material present in manure obtained from animals which are maintained on a diet consisting of a large amount of plant material. It also is concluded that the same attractant which is present in these materials is also present in common, household garbage. The identity of the attractive compound is believed to be the meta-isomer of xylene and has been found to have been concentration dependent. All results obtained from experiments in this project indicate a range of favorable concentrations on the order of five to eight parts per million, with the optimum concentration not having been determined, at present. It has been concluded that much further testing is needed to determine this optimum concentration within closer limits.

The original purpose of this project has been accomplished.

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THE ISOLATION AND IDENTIFICATION OF A NATURAL FLY ATTRACTANT

by

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ABSTRACT

The Isolation And Identification

Of A Natural Fly Attractant

Flies have been known carriers of disease for many years. Methods of control employed in the past, and to some extent in the present, make use of commercially manufactured insecticides. This has caused concern among those people studying the environmental impact of such insecticides. The fly populations of the world must be controlled in order to prevent widespread transmission of the diseases known to be carried by flies. The spreading of disease is not the only problem associated with high concentrations of flies. It is known that milk production and weight increases of cattle are lowered as a result of the bothersome attack on these animals by flies. It was for the purpose of the isolation and identification of the natural fly attractant found in cow manure for a particular species, the face fly, that this research project was undertaken.

The maintainance of a regulated diet in which a large proportion of hay is used, by the Artificial Breeding Station, an extension service of Kansas State University, allowed for the acquisition of manure samples which contain consistant amounts

of the attractant material, producing data which was quite reproducible in its attractive ability for face flies.

The work involved, (a), the formulation of a method for the trapping of the volatile materials responsible for the attraction of flies to the manure, (b) a method for introduction of the volatile materials into the gas chromatographic column, with subsequent separation of the compounds, (c) the isolation of the particular compound responsible for the fly attraction, (d) identification of this compound through various instrumental techniques, and (e) the testing of the isolated compound for effectiveness in fly attraction.

A porous, polymer packing material, commercially marketed under the trade name, Tenax-GC, by Applied Science Laboratories, was used as volatile component adsorbant and also as gas chromatographic column packing material. Separation of volatile components was accomplished through the use of a gas chromatographic column, with the instrument operated in programmed temperature mode. Identification of the component determined to be responsible for fly attraction, by testing with female face flies known to be at the oviposition stage, was accomplished through the usage of mass spectrometry, nuclear magnetic resonance spectroscopy, and by spiking

methods in gas chromatography as a further substantiation of identity.