FRACTIONATION OF THE COMPONENT (S) RESPONSIBLE FOR SEX ODOR IN PORK

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ABSTRACT

FRACTIONATION OF THE COMPONENT(S) RESPONSIBLE FOR SEX ODOR IN PORK

by Harris Bradford Craig

An objectionable odor, commonly referred to as sexual or boar odor, is produced on heating the flesh from most boars and stags. It is described as onionlike, unpleasantly perspirative, or sickly sweet. In federally inspected plants about 3,000 animals are condemned in the United States each year because of sex odor.

Little information is available as to the nature of the odor. Therefore, preliminary studies were undertaken to ascertain if the component(s) responsible for sex odor was/were present in fat, lean or both and to study the solubility properties. Finally attempts were made to collect, isolate and identify the responsible agent(s).

Boar tissues and organs, as well as water and ether extracts of lean and fat, were heat tested for sex odor. Lean tissue was freeze dried; the fat was extracted, and the various fractions were heat tested. Bellies from boars were cured by accepted curing methods and examined for the presence of sex odor after zero, 90 and 120 days of cooler storage. Nitrogen determinations were made on solvent rendered fat from both boars and barrows.

Various distillation and fractionation procedures were employed in an attempt to isolate the agent(s) responsible for sex odor. Barrow fat was similarly treated and served as a control. Distillation methods included: low temperature - high vacuum, high temperature - atmospheric pressure and steam distillation. The volatiles produced by the various distillation techniques were collected in traps immersed in dry ice - ethanol or in ice

water. The contents of the traps were checked for sexual odor by the heat test and the volatiles were separated by gas chromatography.

Fat from boars and barrows was saponified at room temperature using sodium ethylate. The unsaponifiable fraction was heat tested and separated by gas chromatography. In addition, separation by paper and silicic acid column chromatography was attempted.

Sex odor could not be detected until a temperature of 100 to 108 degrees C. was achieved, indicating that the odor component(s) were not volatile under 100 degrees C. or that their formation was heat dependent. Sex odor was not present in the fat-free lean nor in water extracts of lean and fat. The component(s) responsible for sex odor was/were soluble in ether and other fat solvents and appeared to be associated with the fatty tissues.

Appreciable quantities of ammonia were given off by both intact boar and barrow fat, but on rendering the quantity was greatly reduced indicating that connective tissue was the source of this compound. The similarity in nitrogen content of rendered boar and barrow fat indicated that sex odor is probably not due to nitrogenous compounds.

The agent(s) responsible for sex odor in pork was/were not obtained in recognizable form with the distillation methods used in this study. Gas chromatographic analysis of the contents of the traps failed to show any consistent and reproducible differences between volatiles obtained from boar and barrow fat.

Sexual odor was produced when the unsaponifiable fraction of boar fat was exposed to heat, but no reproducible differences were noted between boar and barrow fat on gas chromatographic analysis. Identification of two components from the unsaponifiable fraction was accomplished by using retention times from the gas chromatograph and/or various qualitative tests. Cholesterol and squalene were found to be present in the unsaponifiable fraction of both boar and barrow fat, but these compounds did not produce sexual odor when heated alone in the pure form. Attempts to identify the unsaponifiable component(s) responsible for sex odor by formation of their derivatives and determination of their melting points were unsuccessful.

Three fractions were obtained from boar unsaponifiables when separated by silicic acid column chromatography. The fraction eluted with ethyl ether was the largest and contained most of the sex odor. Gas chromatographic analysis of this fraction indicated that it contained essentially the same components as the unfractionated unsaponifiable matter.

FRACTIONATION OF THE COMPONENT(S) RESPONSIBLE FOR SEX ODOR IN PORK

by

Harris Bradford Craig

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INTRODUCTION

During the past several years considerable effort has been directed toward isolation and identification of flavor and odor components present in various foodstuffs. Prior to this time advances were, of necessity, slow and difficult due to lack of adequate research instruments. With the development of research methods such as gas chromatography, infrared spectroscopy, and mass spectrometry the problem of flavor research has become much less tedious and time consuming. Solutions to seemingly impossible problems have been obtained with relative ease.

Meat is one of the food commodities that has been the subject of extensive flavor investigations. Studies on beef flavor have been undertaken largely in regard to isolation and identification of compounds produced when beef is irradiated. Such flavor might be referred to as an "induced" flavor and is quite objectionable to certain individuals. As contrasted to "induced" off flavor, some meat animal carcasses possess what might be called "natural" off flavors. Since pork and mutton are claimed by some to have objectionable odors, they can be placed in this category. The problem of mutton flavor has not been investigated to any great extent, but studies are currently underway (Jacobson 1960) to identify some of the responsible components.

Off odor in pork, more commonly referred to as sex odor or boar odor, has been a plague to the meat processor for many years. Currently, about 3,000 boars and stags are condemned under Federal Meat Inspection each year in the United States because of sex odor. Probably a much larger number go undetected. Consequently, many hog slaughtering and pork processing establishments avoid slaughtering boars or processing boar carcasses because of the commonness of sex odor. Sex odor has been described variously as pungent, sickly, onionlike, or unpleasantly perspirative. According to Self (1957) it is not confined to boars, but is present in stags, cryptorchids, gilts, barrows, and sows as well. Self (1957) also reported that only a certain percent of the carcasses checked actually possessed off odor. Some were completely free of any trace of sex odor, as detected by exposing a tissue sample to a heat test, which caused the odor to volatilize. Percentagewise, the number of offenders was approximately the same with sows and gilts as for barrows and boars.

In view of the annual economic loss due to condemned carcasses and the adverse processor-consumer relationship, when meat and meat products possessing sex odor are utilized, it was deemed important to study the cause of sex odor in pork. The primary objectives of this investigation were to determine if sex odor was present in pork fat, or lean, or both; to study its solubility characteristics; and to attempt to collect, isolate and identify the responsible component(s).

REVIEW OF LITERATURE

SOME FLAVOR AND ODOR PROBLEMS IN FOODS

Flavor and/or odor of foods are extremely important from the standpoint of consumer acceptance. A food might rate high in every aspect except flavor, but because flavor is lacking or is objectionable, the consumer hastily refuses the product. Flavor was defined by Crocker (1937) as that property of a food or beverage that makes it excite the senses of taste and smell. Kramlich (1959) emphasized the importance of flavor and pointed out that little is known concerning its chemical nature or contributing components.

Vegetables

Since off odors and flavors make a product unacceptable, a number of workers have made a study of these undesirable traits in vegetables. Sondheimer *et al.*, (1955) stated that the bitter flavor present in some carrots could be removed for study by hydrocarbon extraction. In a study of bitter flavor in canned carrots, Shallenberger *et al.*, (1960) divided the flavor into volatile and nonvolatile fractions. The bitter volatile fraction was present in very low concentration and could be removed by steam distillation. After extraction of the distillate with ethyl ether and evaporation, an oil having a bitter, harsh, hot flavor was noted. It was postulated that the volatile component was an essential oil made up of a single sesquiterpene or a mixture of sesquiterpenes.

It is a well known fact that certain vegetables must be blanched before freezing. Lee *et al.*, (1955) showed definite development of off flavor in two to four weeks in frozen unblanched peas, corn, and snap beans. The crude lipid matter extracted from these vegetables showed a definite increase in acid after one week's storage. The increase continued throughout the storage period, but was more pronounced during the early months of storage.

Spencer and Stanley (1954) found that tomato odor was at least partly due to alcohols, carbonyls, and unsaturated compounds. They further noted that changes in the unsaturated compounds may be involved in flavor deterioration of stored tomato products.

Juice

Blair *et al.*, (1952) stated that the characteristic orange-like flavor of orange juice was due to the presence of a small amount of peel oil. Without peel oil, the juice was relatively insipid. When canned orange juice was heated to a temperature higher than 70 degrees F., an off flavor developed due to some substances produced by chemical interaction of the terpenes of the peel oil with the acid in the juice.

Coffee

Coffee bean flavors and aromas are products of a number of treatments such as roasting, storing, and brewing. Kaufman (1951) stated that the crude protein fractions of the coffee bean seemed to be the major source of coffee aroma. Off flavors were due to caffeine or to pyridine which was formed during roasting from trigonelline. Johnson *et al.*, (1938) in a study of roasted coffee found the following constituents: diacetyl, methyl acetylcarbinol, furan, furfuraldehyde, furfuryl alcohol, acetaldehyde, pyridine, and hydrogen sulfide. They postulated that stale flavor of coffee was probably concerned with changes in the volatile aroma and flavor substances and did not involve fat rancidity. Segall and Proctor (1959) noted a relationship between the content of

the mercaptans in a coffee brew and the organoleptic rating of these brews. They suggested that the content of the mercaptans in the coffee brew was closely associated with flavor and flavor changes.

Milk and Milk Products

Milk and milk products are subject to a variety of off flavors. Patton et al., (1957) in a review article on flavor of milk and its products stated that flavor control was concerned with (a) prevention of off flavor, (b) maintenance of desired flavor and (c) development of characteristic flavor. They emphasized that feed flavors which gain entry to milk through the digestive tract of the cow represented the most important off flavors in market milk. Other important flavor defects of milk were termed: oxidized, rancid, and sunlight. The oxidized flavor was due to a series of unsaturated aldehydes which arose through autoxidation of the milk lipids. Rancid flavor was due to hydrolytic splitting of milk fat. Butyric acid was known to be partly responsible for this flavor. According to these authors, hydrogen sulfide, a compound which arose from heat denaturation of milk serum proteins, was evident in milk after momentary heating to approximately 170 degrees F.

Oxidation in relation to off flavor has been studied by a number of workers. Thurston (1937) stated that the most important chemical changes causing off flavors in milk and milk products were due to oxidation involving butterfat or constituents of milk closely associated with butterfat. Forss et al., (1955a) described oxidized flavors in milk as "cardboard", "oily", "metallic", "tallowy", and "emery". They recovered a number of carbonyl compounds from oxidized milk and deduced that they were produced by the preferential oxidation of di- and polyunsaturated fatty acids. In a subsequent paper, (Forss et al., 1955b), it was claimed that the two main constituents of oxidized (cardboard) flavor in milk were 2 - 4 heptadienal and 2 - 4 nonadienal.

Patton (1954) investigated the mechanism of sunlight flavor formation in milk and listed methional as a compound of importance. He did not show the presence of this compound in milk, in which naturally induced sunlight flavor was evident, but it was shown by means of infrared spectral data that methional was a product of the sunlight catalyzed reaction between methionine and riboflavin. He concluded that the amino acid methionine is a specific source of the flavor. Patton _et_ _al_., (1956) studied the relationship between methyl sulfide and milk flavor and stated that a high concentration of this compound could account for certain "cowy" and feed type odors; whereas, abnormally low amounts of methyl sulfide, such as might occur in concentrated or dried milk products, may be responsible in part for the lack of so called fresh milk flavor.

Keeney and Patton (1956) involved delta-decalactone as the agent responsible for the coconut-like off flavor, which developed in butter oil during storage or during heating. Patton and Tharp (1959) studied the effects of steam distillation or saponification on milk fat and identified a homologus series of ketones (acetone through pentadecanone - 2) in milk fat so treated. Fresh milk fat was found to be devoid of such ketones with the exception of acetone. The authors stated that trace amounts of methyl ketones can alter the flavor of milk products.

Flavor and odor problems arise when milk and milk products are exposed to ionizing radiations. Bierman _et_ _al_.,(1956) found that radiation-induced off flavors in milk and cream increased with increasing moisture and fat content. However, as the total solids increased, the product became less susceptible to flavor change. Day _et_ _al_., (1957a,1957b) stated that milk can be sterilized readily by ionizing radiations, but off flavors and odors resulting from this treatment make the product unpalatable. They further noted that of the carbonyls produced in skim milk by irradiation, only acetaldehyde seemed to be of flavor significance (malty). They concluded that the principle radiation induced off flavor appeared to arise from a group of potent, disagreeable smelling sulfur compounds. Methyl mercaptan, methyl sulfide, and methyl disulfide were postulated to be the dominant members of this group. These compounds primarily originate from the milk protein, casein.

Meats

As with milk and milk products, ionizing radiation also produces objectionable flavors and odors in other foods. Proctor et al., (1955) reported that high levels of cathode ray treatment may induce changes in food flavor, that are difficult to mask or modify so that the food will be organoleptically acceptable. Similar results were noted by Pratt and Ecklund (1956). These authors studied the effect of irradiation on several meat items and vegetables. Groninger et al., (1956) evaluated the organoleptic properties of beef, pork, cured ham, salmon, tuna, and halibut and found significant flavor changes in the irradiated product.

Witting and Batzer (1957) prepared a crude reaction mixture of methional from methyl mercaptan and acrolein. They stated that this mixture had an odor typical of ground beef, which had received 2 - 4 megarep of gamma irradiation.

Pearson et al., (1959) stated that the most critical problem in connection with the acceptance of irradiated meats is the development of a characteristic flavor, commonly referred to as "irradiation flavor". Their study indicated that hydrogen sulfide, methyl mercaptan, and carbonyls were responsible for a considerable part of the poor acceptability of irradiated beef, pork, and veal. Similar results were reported by Hedin et al., (1960). They reported that the irradiated or "wet dog" odor was most prominent in an undialyzed meat slurry. When a sample was dialyzed, the most intense irradiated odor was produced by the nondialyzable fraction. It was postulated that the odor was associated with sulfhydryl or closely related compounds, since cysteine and methionine could no longer be detected in the protein after irradiation and since the odor was quenched by sulfhydryl reagents. Batzer and Doty (1955) also implicated sulfur compounds as the source of some undesirable odors in irradiated meat.

Batzer et al., (1957) reported that meats with a high fat content do not develop off odors to the same extent as do leaner meats. Their results indicated that carbonyl compounds produced in irradiated ground beef muscle differed from those obtained from irradiated beef or pork fat. The amounts of these compounds produced were shown to increase with increasing irradiation dosage.

Animal flesh is known to acquire off flavor and odor from certain rations. Roine et al., (1953) fed spoiled meat scraps and horse manure to growing pigs. Taste and odor tests of the flesh showed that the meat scrap group had taken on repulsive tasting and smelling substances. This was especially true of the lard where more volatile aldehydes, methyl ketones, secondary amines, trimethyl amine, skatole, primary amines, and phenols were found as compared to lard from control pigs. Kemp et al., (1952) and Grummer et al., (1950) examined the flesh of hogs which had been treated with benzene hexachloride prior to slaughter and found that this compound produced off flavors and odors when applied immediately before death. Both the lean and fat were involved.

Vacuum packed, stored, dehydrated pork developed an off flavor and/or odor during storage at elevated temperatures and/or for long time intervals (Burnett et al., 1955). These authors reported that the volatile constituents obtained from this product were basic in reaction and exhibited reducing properties. Acetaldehyde was present in dehydrated pork samples stored at both 20 degrees F. and 94 degrees F., whereas, ammonia was detected in meat samples stored at 100 degrees F. and 160 degrees F.

The problem of sex odor in pork has been recognized for some time. However, there is little information available as to the nature of the odor and the agents responsible for its production. Lerche (1936) reported that as soon as the male hog was sexually mature and the testes became capable of functioning, there appeared a specific sexual odor in the meat and fat of the animal. He described the odor as being onionlike or unpleasantly perspirative and claimed that it occurred in all boars with normally developed testes and in cryptorchids, unless the testes lying in the abdominal cavity were atrophied. He stated that the tissue must be heated and the vapors smelled in order to detect the odor. This was accomplished by placing small pieces of tissue in water in an Erlenmeyer flask and boiling. The vapors were allowed to bathe the nostrils of the tester. Tissue was also heat tested by frying in a skillet. When the heated tissue had cooled, no odor was evident. He also noted that after male hogs had been castrated, the sexual odor disappeared from the tissue. The tissue was found to be devoid of odor 57 - 68 days post-castration.

Gereke (1936) postulated that a connection must exist between the testes and the parotid salivary gland which is located near the ear.

According to this author, cryptorchid boars may often be detected by an over production of saliva. This condition was also known to occur when boars are sexually excited. The parotid gland, according to him, was a good tissue in which to detect the odor. He stated that the odor was not dependent upon the size of the testes, but more upon the condition of the animal. He noted sex odor to be especially evident in the case of small, thick-skinned animals, which were lacking in fat and retarded in development.

Kunze (1936) claimed that the tissue of cryptorchid boars, which had been condemned because of too strong a sexual odor could be freed of this odor by "pickling". After pickling for three weeks, the tissue was completely free from odor. He claimed that the odor producing material(s) had been bound by the salt.

Miller (1958) outlined the procedure to be followed in federally inspected plants. Stags and boars should be separated from the regular kill during antemortem inspection and identified for checking for sexual odor on post-mortem examination. The lean and fat are then tested for odor by heating. A carcass which exhibits sexual odor should be condemned as unfit for food. Miller (1958) grouped carcass odors into two categories: (a) those traceable to material ingested by the animal and (b) the odor known as sexual odor in swine. Sexual odor is usually detected in carcasses of boars and recently castrated stags. He further points out that care should be exercised not to confuse this condition with odor which is imparted to a portion of the carcass by contamination with smegma from the prepuce.

Howe and Barbella (1937) claimed that occasionally during cooking, meat from very old animals, such as bulls, cows, or rams developed an extremely unpleasant odor, often characterized as ammoniacal. They also reported that sexual odors in tissues were usually associated with boars and stags that had been slaughtered soon after castration.

The origin of the usage of the words "sexual odor" is not known. Since the odor has been shown to disappear several weeks post castration, it would seem to be sex linked in some way and hence the term "sex odor." In view of this fact, the implication of androgenic hormones has been postulated. Bratzler et al., (1954) found that meat from boars was definitely inferior from a palatability standpoint. Cooking of pork chops from boars by braising produced a definite "off" or "boar" odor, which according to them carried through to the cooked product. No objectionable odor was detected in the chops from 180 pound pigs castrated 34 days before slaughter. One group of pigs was castrated 118 days before slaughter and implanted with a 193 milligram pellet of testosterone propionate at the time of castration. Examination of the heated tissue from these pigs failed to show any evidence of sexual odor.

Johnston et al., (1957) fed two levels (9 or 15 mg. per lb. of feed) of methyl testosterone to growing fattening pigs and found no objectionable odor in the flesh of pigs fed this androgen. Perry et al., (1956) obtained similar results when methyl testosterone was fed to pigs of similar weights and ages.

Christian and Turk (1958), in a study of the cause of sexual odor in the boar, designed an experiment using the following groups of pigs: 1. untreated boars, 2. untreated barrows, 3. boars with the preputial diverticulum removed, 4. barrows injected subcutaneously with 20 milligrams of testosterone daily, 5. boars fed 10 milligrams of stilbestrol daily, and 6. boars fed 50 milligrams of stilbestrol daily. Boar odor and flavor were determined by panel evaluation of the boneless roasted loin. Results indicated that the panel could detect the most odor in group 1 followed by groups 3, 5, and 4, in that order. Panel scores on samples from the above lots, which had been frozen and stored for five months, were generally somewhat lower. This indicated that the odor had diminished during freezing and storing.

Probably one of the most extensive studies concerning the problem of sex odor in pork was conducted by Self (1957). His work was stimulated by the research department of Oscar Mayer and Company. This organization had received consumer complaints regarding a "boar odor" in pork. This odor was also noted along their kill line, although they do not slaughter boars. Tissue samples were taken from the diaphragm muscle of 343 pigs as they passed down the kill line. Samples were wrapped in aluminum foil and heated to approximately 200 degrees F. under infrared tubes. Testing was performed by breaking open the foil wrapper and allowing the volatile substances to bathe the nostrils of the tester. Odor intensity was classed as strong, medium, or none. The incidence of the odor (17 per cent of those checked) was found to be as high among female hogs as among castrated males. Another group of older female hogs in various stages of the reproductive cycle was examined for odor. Approximately 17 per cent of the group total had sexual odor, but there was no clearcut group of offenders when classified on this basis. Progesterone administered to 22 gilts did not appear to alter the incidence of the odor in this group. In a group of 31 sexually mature boars a total of eight, or 25.8 per cent had some sex odor. A study of boars by breed failed to show that any genetic group had a greater incidence of the odor than any of the other breeds tested. The author concluded that on the basis of data presented it seems that

sex and breed have little influence on the overall incidence of sex odor or boar odor in pork.

Dutt et al., (1959) removed the preputial diverticulum from boars and found that the characteristic ranting odor was reduced, but at ten months of age no difference in boar odor was found between carcasses from operated and unoperated boars. Lean areas were free of the odor, but all fat areas sampled gave off boar odor when heated. The greatest concentration was found in the fatty tissue of the prepuce where brownish orange areas were noted in the dorsal and lateral regions of the orifice. Histological examination showed this tissue to be modified sebaceous glands containing colloid-like material. Similar glands in barrows appeared to be non-functional when examined histologically. Alcohol-ether extraction of the body tissues showed that the agent responsible for the odor is lipophilic and these authors postulated that it is a muscone. These workers surgically removed the glandular area in five immature boars. The animals were slaughtered at weights varying from 290 to 350 pounds. No odor was detected in any portion of the carcasses of two boars from which the preputial glands had been completely removed. Some odor was noted in carcasses of boars, in which post-slaughter examination revealed that not all of the glandular area had been removed. It was concluded that the preputial glands, which are dependent upon the male sex hormone for secretory activity, produced a fat diffusible material which was responsible for sexual odor in boar carcasses.

Mutton Flavor

Certain individuals object to the smell and taste of lamb and mutton. Ziegler (1958) and Kean (1959) pointed out the fact that mutton flavor and odor does exist. Undoubtedly this has an effect on lamb and mutton consumption.

The problem of mutton flavor has not been explored from the standpoint of volatile components. Jacobson (1960) is currently working on identification of the components of flavor in lamb and mutton and application of this information toward increased utilization of these meats. McInnes *et al.*, (1956) found that the steam volatile acids of mutton fat, which might have some influence on flavor, included those from formic (C_1) to an acid with ten carbon atoms. Isobutyric, isovaleric, an anteiso acid, and alpha methyl butyric acid were also found. This was the first time, according to the author that these four compounds, as well as formic acid, have been shown to be components of an animal depot fat.

METHODS EMPLOYED IN THE SEPARATION, CONCENTRATION, AND IDENTIFICATION OF FOOD FLAVOR AND ODOR COMPONENTS

Chemical

One of the most helpful tools the researcher has at his disposal is the use of chemical qualitative tests. Since a number of the volatile flavor and odor components of foods have been shown to be carbonyls, the most logical approach to the identification of these materials is by use of 2 - 4 - dinitrophenylhydrazine. This reagent, when united chemically with a carbonyl, forms the corresponding 2 - 4 dinitrophenylhydrazone. Henze *et al.*, (1954) employed this reagent for reaction with carbonyl compounds obtained from apple storage volatiles. A total of 18 compounds were obtained and these were separated by silicic acid chromatography and analyzed by use of a Beckman D. U. spectrophotometer. Wong *et al.*, (1958) used this method in studying methyl ketones obtained from evaporated milk. Dutra *et al.*, (1959) also found this procedure was appropriate for studying the carbonyls obtained from evaporated milk. The 2 - 4 dinitrophenylhydrazones were identified by melting point determination and paper

chromatography. Methyl ketones formed from milk fat during steam distillation and saponification were likewise identified as their 2 - 4 dinitrophenylhydrazones by Patton and Tharp (1959). Similarly, Patton et al., (1958) used this method for separation and aid in identification of the volatile carbonyls from cheddar cheese.

Pippen et al., (1958) obtained 18 carbonyl compounds from a simmering mixture of chicken meat and water by passing the volatile stream through a solution of 2 - 4 dinitrophenylhydrazine. The corresponding hydrazones obtained were separated by use of column chromatography. Kramlich (1959) also used 2 - 4 dinitrophenylhydrazine as an aid in identification of the carbonyls in beef flavor volatiles. The hydrazones were chromatographed using paper.

Dimick and Makower (1956) reported on the use of 2 - 4 dinitrophenylhydrazine as a method for determining the volatile carbonyls of strawberries. Carbonyls were estimated by converting to their 2 - 4 dinitrophenylhydrazone derivatives, and forming colored complexes with these by addition of potassium hydroxide to an alcoholic solution of the hydrozone. Monocarbonyls produced a red color, whereas dicarbonyls such as biacetyl were recognized by the appearance of a blue color.

The organic and inorganic sulfur compounds present in a food, or those generated when it is cooked, are capable of imparting undesirable odors to the product. Kohman (1952) claimed that the characteristic onion oder (flavor) was due to allyl propyl disulfide which was liberated by an enzyme when the raw onion cell was ruptured. This compound was steam distilled from the rest of the onion, oxidized to sulfate sulfur with bromine, and determined by gravimetric or colorimetric means.

Dateo *et al.*, (1957) trapped the volatile sulfur components from cooked cabbage employing a train of traps filled with anhydrous calcium chloride, lead acetate, four per cent aqueous mercuric cyanide and three per cent aqueous mercuric chloride. This trapping system removed water and aided in separation of organic and inorganic sulfur compounds.

Pippen and Eyring (1957) characterized the volatile sulfur fractions of cooked chicken broth. Hydrogen sulfide was determined by the methylene blue method, which is specific for sulfide sulfur. The quantity of hydrogen sulfide was determined colorimetrically, and total sulfur was determined gravimetrically as barium sulfate.

Patton *et al.*, (1956) trapped methyl sulfide from the air space in a cold wall milk tank using mercurous chloride (1% aqueous) as the trapping agent. When collection was complete, the sample was treated with an equal volume of normal hydrochloric acid and warmed slightly. The odor given off appeared identical with that of methyl sulfide. Lead acetate was employed by Kramlich (1959) to trap the volatile sulfur compounds obtained from simmering ground beef.

Sliwinski and Doty (1958) determined the amount of methyl mercaptan in irradiated meat by use of N, N - dimethyl - p - phenylenediamine. This reagent forms a red colored complex with methyl mercaptan. The amount of mercaptan present was determined spectrophotometrically. These workers found that hydrogen sulfide interfered with the colorimetric determination of mercaptan, but that it could be removed by precipitation with mercuric acetate. Hornstein *et al.*, (1960) found that chemical identification of beef flavor components represented a satisfactory approach to the problem. They identified several carbonyls, hydrogen sulfide, and ammonia by various chemical means.

Haagen - Smit *et al.*, (1949) identified various classes of chemical compounds present in the volatile constituents of grapes by use of appropriate chemical tests. The volatiles were collected in traps immersed in a dry ice - ethanol mixture. The trap contents were saturated with chemically pure ammonium sulfate and extracted with peroxide free ether. The ether extract, after drying over anhydrous sodium sulfate, was distilled to remove the ether. This ether free liquid was then fractionated and tests performed on the various fractions. Several alcohols, carbonyls, and acids were identified by this method.

Mackay and Hewitt (1959) utilized enzymes present in cabbage and mustard for flavor development of reconstituted dehydrated cabbage. Flavor of these products, due in part to isothiocyanates, was released by treatment with thioglucosidases. Assay for isothiocyanates was made using gas chromatography and paper chromatography. This work emphasized the importance of enzyme liberation of flavor components.

Olsen *et al.*, (1959) determined ammonia in a distillate of several processed meats by use of Nesslers' reagent. Appropiate tests for other amines yielded negative results. Bouthilet (1951) identified ammonia as a constituent of chicken flavor by reaction of a steam distillate of chicken meat with benzene sulfonyl chloride. This reaction produced the sulfonamide of ammonia.

Column and Paper Chromatography

Kramlich (1959) and Hornstein *et al.*, (1960) used paper chromatography to separate and identify the 2 - 4 dinitrophenylhydrazones obtained by reacting volatile carbonyls from beef with 2 - 4 dinitrophenylhydrazine. Likewise, Pippen *et al.*, (1958) and Pippen and Eyring (1957) have studied the flavor volatiles from cooked chicken using column and paper chromatography and ion exchange chromatography, respectively.

Clements and Deatherage (1957) and Lentner and Deatherage (1959) employed both column and paper chromatography in studies of the volatile components of roasted coffee. In addition, some non-volatile constituents were separated and identified by use of these methods. Various methyl ketones and other flavor compounds from evaporated milk and from milk fat have been separated and identified by use of column and paper chromatography (Wong et al., 1958; Patton and Tharp, 1959; and Dutra et al., 1959).

Silicic acid chromatography was employed by Dimick and Makower (1956) for identification of carbonyls obtained from strawberry essence and by Henze et al., (1954) for the determination of carbonyl compounds present in apple storage volatiles.

An assay method for mustard oils in cabbage and mustard, outlined by Mackay and Hewitt (1959), employed paper and gas chromatography in an effort to determine the amount of oils in these products.

Distillation

Numerous workers have employed various distillation techniques as an aid in the separation of food flavor components. Dateo et al., (1957); Pippen et al., (1958); and Kramlich (1959) utilized distillation at atmospheric pressure for the separation of volatiles from cabbage, chicken, and beef, respectively.

Steam distillation has been accepted as an excellent technique for distillation of certain substances. This method allows components which have boiling points higher than water to be distilled from an aqueous mixture. Dacre (1955) used it for a study on cheddar cheese. Kohman (1952) noted it was a satisfactory method for removal of allyl propyl disulfide from onion. He found that a temperature of 121 degrees C. was necessary. Olson et al., (1959) obtained some of the volatile flavor components from processed meat by use of steam distillation. The condensables were trapped in special glass traps immersed in ice water.

Patton and Tharp (1959) found that steam distillation of milk fat produced a number of methyl ketones. These and other volatiles were trapped in two dry ice - ethanol traps. They maintained a system pressure of less than one millimeter and a temperature of 200 degrees C. Dutra et al., (1959) removed some flavor compounds from evaporated milk by steam distillation. The steam distillate was concentrated about 40 to one in a modified Oldershaw - bubble plate column.

Bouthilet (1950) employed a low pressure steam distillation apparatus to separate volatile chicken flavor components from broth. In a later paper (Bouthilet 1951) he found that this method was suitable as an aid in fractionation of chicken flavor constituents.

In view of the fact that many compounds are altered when heated to the temperatures necessary in the preceding distillation methods, a number of workers have resorted to high vacuum - low temperature distillation - (Haagen - Smit et al., 1949; Patton et al., 1958; Merritt et al., 1959; Hornstein et al., 1960; Stahl, 1957; Niegisch and Stahl, 1956; and Wong et al., 1958). Most of these workers employed cold traps of various temperatures to trap the distillates. In some cases, the components collected were separated by taking advantage of their volatility at different temperatures.

Johnson and Frey (1938) distilled ground dry coffee under high vacuum, gradually increasing the temperature to 100 degrees C. Some receivers for collection of the volatiles were cooled in solid carbon dioxide and some in liquid air. Lockhart (1957) also used dry vacuum distillation, but the product was not heated.

Dimick and Makower (1951) described a laboratory scale continuous vacuum flash evaporator, a type of distillation apparatus. With this unit a juice or puree was concentrated and a water solution of volatile flavor components was obtained.

Olson *et al.*, (1959) removed some of the flavor components from defatted samples of heat processed beef luncheon meat with methanol. This is an example of solvent extraction of flavor components. Where this method is used, the solvent is generally removed by distillation or evaporation.

Gas Chromatography, Mass Spectrometry, and Infrared Spectroscopy

Gas chromatography probably evolved from a study made by Martin and Synge (1941) who used two liquid phases rather than a gas phase and a liquid phase. Since that time, the method has been used for a variety of separation and identification problems (James, 1952; James and Martin, 1952; Ray, 1954; and James and Martin, 1956).

Since its inception, this method has been employed for a number of flavor and odor problems. The components of the gaseous emanation products of the onion were examined by Niegisch and Stahl (1956) using gas chromatography, mass spectrometry, and infrared spectroscopy. They identified a total of six compounds. Bernhard (1958) found a number of compounds present in cold pressed lemon oil by the use of gas chromatography and infrared spectroscopy. Mackay and Hewitt (1959) assayed mustard oils from cabbage and mustard by using a gas chromatography instrument equipped with a one foot column. A short column was deemed necessary so as to avoid exposing the oils to heat for an excessive period of time.

Patton *et al.*, (1958) used a combination of gas chromatography and mass spectrometry to separate and identify the volatile components of cheddar cheese. Day *et al.*, (1957b) and Patton *et al.*, (1956) identified some milk flavor components by use of gas chromatography. Tentative identities for a fractional component from the various volatile mixtures were obtained by smelling the component as it emerged from the column and by comparing its retention volumes with those of known compounds. Day *et al.*, (1957b) also employed mass spectrometry as a tool for identification of the volatile components obtained from gamma irradiated skim milk.

Hankinson *et al.*, (1958) found that gas chromatography provided a means for rapid and accurate analysis of the volatile fatty acids in milk. Similarly, Jennings (1957) applied this method to a study of the volatile flavor components present in a distillate of commercial dairy starter. The procedure for proper packing of a column for use in a gas chromatographic instrument was given by both of these authors.

Wynn *et al.*, (1960) reported that gas chromatography could be employed as a means of detecting oders in milk. The claim was made that this method offers a valuable tool for the study of the effectiveness of deodorizing equipment as a means of removing the undesirable volatile flavoring components of milk.

Recent attacks on the chemistry of coffee aroma have been made using gas chromatography (Lockhart, 1957). This author claimed that the technique was applicable to all types of problems involving micro-concentration of odorants. Likewise Rhoades (1958) and Zlatkis and Sivetz (1960) employed gas chromatography as a means of separation and aid in identification of the volatiles obtained from coffee. Volatile mixtures were trapped in dry ice - ethanol or liquid air traps and subjected to analysis by infrared spectroscopy and mass spectrometry as well as by gas chromatography.

Kramlich (1959) trapped the volatile components from simmering ground beef in traps immersed in liquid air, and subsequently analyzed the trap contents using gas chromatography. Infrared spectral analysis was also attempted but failed to yield any definite information. Merritt et al., (1959) separated the volatile components of irradiated beef into three fractions by use of a high vacuum - low temperature distillation assembly. This method makes use of the differences in the vapor pressure - temperature relationships of the compounds present. Two of these fractions were separated by means of gas chromatography and the components identified by mass spectrometry.

Stahl (1957) outlined a procedure for the investigation of an unknown odor. He emphasized the fact that gas chromatography and mass spectrometry are truly synergistic, the former being used as an elegant means for separation of a mixture, so that its components can be identified by mass spectrometry. He claimed that the identification of an unknown can be very difficult, when attempted by gas chromatography alone. The combination of techniques employed by this author were adapted to a variety of flavor problems.

During the past few years, gas chromatography as an analytical method has been greatly improved. Column efficiency has been increased several fold by use of coated capillary columns (Golay, 1958; Zlatkis and Lovelock, 1959). More sensitive detectors have also been developed. The ionization detector described by Lovelock (1958) is claimed to be capable of detecting concentrations of 2×10^{-12} moles of most organic compounds.

EXPERIMENTAL PROCEDURE

The preliminary part of this study was undertaken to determine the tissue location, solubility characteristics, and volatilization temperature of sex odor in pork. In addition, an experiment was conducted to determine if sex odor could be prevented in the carcass by an intramuscular injection of tranquilizer into the live hog. The final phase of this investigation dealt with attempts to collect, isolate, and identify the agents responsible for sex odor in pork.

PRELIMINARY STUDIES

Source of Experimental Material

All boars used in this investigation were procured from the Michigan State University swine farm. Barrow and gilt fat was obtained from hogs slaughtered in the University Meats Laboratory. A considerable amount of the boar fat used was provided through the courtesy of Crown Packing Company, Detroit, Michigan, while the remainder was obtained from boars slaughtered at the Michigan State University Abattoir.

Method of Sex Odor Detection

Tissues were examined for the presence of sex odor by heating small cubes in a skillet or boiling them in an Erlenmeyer flask with a small amount of distilled water. At least three individuals verified the presence or absence of the odor.

Determination of Sex Odor Volatilization Temperature

Lard, free of sex odor, was placed in a deep fat fryer and heated to an initial temperature of 60 degrees C. Boar fat samples were placed in a small amount of water in Erlenmeyer flasks (125 ml.) fitted with cork stoppers. A short length of glass tubing was inserted into the stoppers and the flasks were partially immersed in the lard. The temperature of the lard was increased gradually and the heat equalized by agitating with an electric stirrer. As the vapor emerged from the glass tube, the temperature was recorded at which sex odor was first noted.

<u>Examination of Boar Tissues and Organs for the Presence of Boar Odor</u>

An 18 month old Yorkshire boar was slaughtered in the University Meats Laboratory. Various tissues and organs were removed for use in determining the location of sex odor. The carcass was boned and the lean and fat separated, frozen, and stored in a freezer at - 20 degrees F. Barrow lean and fat were similarly treated and used as controls.

The following boar tissues and organs were examined for sex odor by use of the heat test described previously: lean from loin (devoid of visual fat), heart, parotid gland, liver, lung, kidney, fat from the diaphragm, spleen, testes, Cowpers gland, seminal vesicles, sperm cord and its overlying muscle, penis, diaphragm muscle (free of visual fat), tongue, and preputial diverticulum.

A sample of lean tissue from the loin of a boar was ground twice in a food chopper and blended with five parts of distilled water for three minutes in a Waring blender. The resulting slurry was centrifuged for 20 minutes at 2100 revolutions per minute. After centrifugation, the supernatant was decanted, strained through cotton, and heated over a gas flame to determine the presence or absence of sex odor. The residue was removed from the centrifuge bottle by adding distilled water and stirring. It was subsequently tested for odor by heating. The lean tissues from a barrow were similarly treated.

In order to determine the effect of water extraction on fatty tissue, a fat sample from a boar and fatty tissues from a barrow were blended with five parts of distilled water for three minutes. The resulting emulsion type slurry was centrifuged for 20 minutes at 2100 revolutions per minute.

The fat layer was broken and the aqueous extract strained through cotton. Both the fat layer and the aqueous extract were heated to ascertain the presence or absence of sex odor.

Effect of Curing Boar Meat on Intensity of Sex Odor

The two bellies from the Yorkshire boar were rubbed with three-fourths of an ounce of a curing mix per pound, which consisted of eight pounds of salt, two pounds of sugar, one ounce of sodium nitrate, and two ounces of sodium nitrite. After pressure curing for 14 days, the bellies were soaked for 30 minutes in water and dried. One bacon slab was stored in a cooler at 38 degrees F.; the other, after smoking, was stored similarly. Samples from both slabs were heat tested in a skillet after zero, 90, and 120 days of cooler storage.

The Presence and Solubility Properties of Components Responsible for Sex Odor

A frozen 100 gram sample of lean from the ham of a boar was shaved into small pieces and placed in a round, 500 milliliter flask. Moisture was removed from the sample by freeze drying for 26 hours employing a glass receiver immersed in a dry ice - ethanol mixture. The vacuum was produced using a Cenco Hyvac 7 vacuum pump. The frost which collected in the receiver was allowed to thaw and was then heated to determine the presence or absence of sex odor. Similarly, some of the dried lean was rehydrated with distilled water and checked for sex odor. The remaining portion of the freeze dried lean was placed in a Goldfisch fat extractor and extracted for three hours using anhydrous diethyl ether as the solvent. After extraction, the moisture-free, fat-free lean was rehydrated with distilled water and heated to check for the presence of sex odor. The extracted fat was heated in a small beaker over a gas flame and the volatiles coming off smelled to determine the presence or absence of sex odor.

In addition to the above procedure, samples of fat and lean from both boars and barrows were cubed, placed in 250 milliliter Erlenmeyer flasks and heated gently over a gas flame. The ensuing vapors were checked for the presence of sex odor.

The solubility characteristics of the sex odor component(s) toward water and several organic laboratory solvents were determined. Extraction of boar fat and lean with water was conducted according to the method mentioned previously. Extraction of boar fat with organic solvents was accomplished by placing 200 grams of ground fatback in a Waring blender and blending for one and one-half minutes with an equal quantity of solvent. The blended samples were filtered first through two layers of cheese cloth and then through Whatman 41-H filter paper. Water accumulated during the above process was removed by use of a separatory funnel. Solvents were removed by heating the solvent-fat mixture on a steam bath (180° F. or less) until the odor of the solvent was no longer evident. The amount of extracted solvent-free fat was noted, and a portion was heated to determine the presence or absence of sex odor. The following organic solvents were employed: acetone, carbon tetrachloride, chloroform, diethyl ether, petroleum ether (B. P. 30 - 60° C.), dioxane, and ethyl alcohol.

Nitrogen Determination of Rendered Boar and Barrow Fat

Wet neutral litmus paper strips were held in the volatile stream from the heated boar and barrow fat and the color change of the paper noted. This indicated whether the vapor was acidic or basic. Since the test indicated a basic reaction, it was decided to conduct nitrogen determinations on the solvent rendered boar and barrow fat. These determinations were made employing the Kjeldahl procedure outlined by the Association of Official Agricultural Chemists (1955).

Saponification

Both boar and barrow fat were saponified as an aid in their fractionation. Deuel (1951) suggested that fat could be saponified in the cold by several methods, one of which was treatment of the fat with sodium ethylate. This method was employed in this portion of the study since the sex odor appeared to be volatile. One hundred grams of boar fat were rendered by blending with 200 milliliters of diethyl ether for one and one-half minutes in a Waring blender. The connective tissue was removed by filtering the ether extracted fat through four layers of cheese cloth. The filtered liquid was placed in a 2000 milliliter Erlenmeyer flask and 400 milliliters of diethyl ether was added. Sodium ethylate was prepared by dissolving 16 grams of kerosene free-metalic sodium in 200 milliliters of 95 per cent ethyl alcohol. Any alcohol that evaporated was replaced. When the sodium had dissolved, the resulting sodium ethylate was added and stirred into the mixture of ether and fat. The flask was stoppered, shaken vigorously, and allowed to remain at room temperature for 24 hours or more. After saponification was complete, the liquid was filtered from the soap by suction filtration using Whatman 41 filter paper and a water aspirator. The soap was extracted once with ethyl ether, refiltered, and allowed to dry at room temperature. The ether filtrate, containing some soap and the unsaponifiable matter, was washed repeatedly with distilled water in a separatory funnel. If the ether and water layers were sluggish in separating, a small amount of ethyl alcohol greatly facilitated separation. If a complete emulsion formed, reagent grade sodium chloride was added to cause separation of the ether and water layers. After washing, the ether extract was dried overnight with anhydrous sodium sulfate, filtered, and reduced to a volume of a few milliliters. Volume reduction was accomplished under vacuum at room temperature. Both the soap and the unsaponifiable matter were heated to determine the location of the sex odor.

Injection of Live Boars with "Diquel" Animal Tranquilizer

This portion of the study was conducted in order to determine the effect, if any, of animal tranquilizer injection on sex odor suppression in live boars, and hence in the carcass. Four mature Yorkshire boars were restrained and a one and one-half by three and one-half inch fat sample taken by biopsy technique from the region of the last rib just off the mid line of the back. Five cubic centimeters of two per cent procaine hydrochloride were used as an anesthetic. After removal of the biopsy sample, Diquel was injected through the biopsy wound into the *Longissimus dorsi* muscle. The data are given in Table 1.

Table 1 Boar weights and amount of tranquilizer administered

Boar number	Weight (lbs.)	Amount Diquel injected (c.c.) *
6 - 4	412	3.50
1 - 6	407	3.75
17 - 9	583	4.50
13 - 11	392	3.75

* Each c.c. contained 50 mg. of ethyl isobutrazine (2 - ethyl - 3' - dimethyl - amino - 2' - propyl) 10 phenothiazine hydrochloride)

METHODS EMPLOYED FOR ISOLATION AND COLLECTION OF SEX ODOR COMPONENTS

Low Temperature - High Vacuum Distillation

Attempts were made to collect the volatile sex odor component(s) from boar fat by a low temperature - high vacuum distillation assembly. The procedure was similar to that outlined by Merritt *et al*., (1959). One hundred and fifty grams of frozen boar fat were cubed and placed in a

round, 500 milliliter flask. The flask was attached to a glass receiver and then immersed in liquid nitrogen. The system was evacuated using a Cenco vacuum pump. After evacuation of the system, the liquid air was removed from the sample container and placed around the receiver. Distillation was continued for a period of six hours. The frost and the dried fat were exposed to heat to check for the presence of odor.

Gas Chromatography of Volatiles Obtained by High Temperature Atmospheric Pressure Distillation

Various modifications of this type of distillation procedure were attempted. Initially, boar fat and skin was placed in a round 500 milliliter flask and heated for 60 minutes by means of an electric mantle. Temperature was controlled by use of a Power-Stat voltage rheostat maintained at a setting of 60 volts. Volatile materials were passed through an air cooled condenser and the vapors trapped in a two normal solution of hydrochloric acid. The acid solution was heated and checked for sex odor. The apparatus was later modified by replacing the air cooled condenser with a short length of insulated glass tubing. Instead of acid, the volatiles were trapped in a glass trap made of an Erlenmeyer flask containing a one-half inch layer of glass beads. The trap was immersed in a dry ice - ethanol mixture. Volatiles obtained in the trap were heated over a gas flame and the odor of the fumes noted.

A distillation system similar to that just described was assembled and the volatiles from boar and later from barrow fat were allowed to bubble through a glass tube immersed in diethyl ether. Heat was applied to the system for a 90 minute period. This procedure was repeated five times using a fresh supply of fat each time. The ether - water mixture was removed and separated by use of a separatory funnel. The recovered ether layer was dried over anhydrous sodium sulfate and reduced to a volume of one milliliter on a steam bath. The resulting solution was analyzed by gas chromatography using a 100 foot capillary column containing dinonyl phthalate or Apiezon "L" as the liquid phase. Liquid samples were injected by means of a ten microliter Hamilton syringe.

Additional distillation studies were conducted in an effort to obtain volatiles from boar and odor free barrow fat, which could be analyzed by gas liquid partition chromatography. A Barber-Colman, Model 20, Ionization Detection System, gas chromatograph was employed for this purpose. The instrument was equipped with a radium detector and a six way gas sampling valve. The latter device was manufactured by the Wilkens Instrument and Research Company, Walnut Creek, California. The design of the Barber-Colman instrument allows the use of packed or capillary columns.

A distillation assembly similar to that employed by Kramlich (1959) was used in collecting the volatiles from boar and barrow fat. A charge of 500 grams of fat with skin was mixed with an equal quantity of distilled water and placed in a three neck, 12 liter flask. The flask was fitted with a glass tube through which nitrogen gas was bubbled. Volatiles were passed through a small glass air cooled condenser and a short length of rubber hose into a glass spiral cold finger trap, which was immersed in a dry ice - ethanol mixture. The contents of the flask were heated for two hours using a gas flame. The volatiles were vaporized by heating in a hot oil bath at 145 degrees C. and were injected into the gas chromatography instrument by means of the six way gas sampling valve.

Results of the above attempt dictated alteration of the assembly. Sample size was increased to about 2500 grams. An ice water trap was added between the condenser and the dry ice - ethanol trap to remove water vapor from the volatiles. Glass beads were used in the ice water trap to increase the area for condensation. The nitrogen stream was replaced by a stream of air from a laboratory supply line. After heating the fat for five to six hours, the trap contents were examined by gas chromatography. The wet ice trap contents were extracted with diethyl ether to obtain a sample suitable for analysis. A five foot, one-fourth inch copper column employing diethylene glycol succinate as the liquid phase and Chromasorb as the stationary inert phase was used for the separation of the volatile components.

Other modifications of the above procedure included the following:

1. Replacement of the gas flame with a hot lard bath to prevent charring of the fat. The lard bath temperature varied between 140 to 150 degrees C.

2. Application of a mantle to the condenser to maintain the temperature at 105 to 110 degrees C., and thus prevent premature condensation of the volatiles.

3. Removal of the skin from the fat before heating to avoid producing volatiles from this source.

4. Removal of the connective tissue from the fat to avoid production of volatiles from this source. Ten pounds of boar fat were ground through a one-fourth inch plate and then warmed slightly in a stainless steel, steam-jacketed kettle. After heating, the slurry was strained through stockinette and two layers of cheese cloth. The connective tissue was discarded and the liquid fat placed in the 12 liter flask and heated as previously described for the intact boar fat.

5. Removal of the wet ice trap to avoid trapping of the volatiles at this point.

6. Gas chromatographic separation of the volatiles obtained from boar fat and barrow fat using a ten foot, one-fourth inch copper column packed with diethylene glycol succinate on Chromasorb.

Since it was postulated that the above described procedures might have exposed the fat to excess heat for a prolonged period of time, a different type of collection assembly was set up. Boar fat without skin was cut into small cubes and 200 grams were placed in a one liter pyrex suction flask. The top of the flask was closed with a cork stopper containing an "L" shaped glass tube. This tube was connected to a 100 milliliter suction flask immersed in ice water which acted as the wet ice trap. This trap was followed by a glass spiral trap immersed in dry ice - ethanol. The flask containing the sample was heated for 30 minutes on an electric stove using medium heat. Volatiles produced by heating were drawn through the traps by a slight vacuum. Components collected in the dry ice - ethanol trap were separated using the ten foot diethylene glycol succinate column previously described. The preputial glands from the fat surrounding the prepuce were also treated as outlined above.

In other studies employing the above procedure, the dry ice-ethanol trap was removed. The flask containing the sample was recharged with 615 grams of fat for a total of ten times. The total aqueous distillate trapped in the wet ice trap at four to five degrees C. was saturated with sodium chloride and extracted with four - 50 milliliter portions of diethyl ether. The resulting ether extract was dried over anhydrous sodium sulfate. After drying, the ether solution was reduced under hydrostatic vacuum to a volume of two-tenths of a milliliter and examined by gas chromatography using the following columns: 100 foot capillary dinonyl phthalate; 100 foot capillary Apiezon "L"; or ten foot, one-fourth inch columns packed with flexol plasticizer, mannitol or silicone 200.

The inert phase used in the packed columns was Chromasorb "W".

Direct Sampling Methods

A 200 gram sample of boar fat was cubed, placed in a 500 milliliter Erlenmeyer flask and was heated on a hot plate. When the sex odor emerged from the flask, a five milliliter hypodermic syringe was inserted into the neck of the flask and a one milliliter sample of the gas drawn into the syringe. This was injected into the gas chromatographic instrument equipped with a 100 foot capillary column using Apiezon "L" as the liquid phase. A ten foot diethylene glycol succinate column was also employed and the gas sample size increased to five milliliters. This portion of the study was repeated and the fat was heated in a skillet on an electric stove rather than in the Erlenmeyer flask.

Steam Distillation of Boar Fat

Chopped boar fat (500 g.) was placed in a two liter double neck round bottom flask. A steam distillation apparatus was assembled and the boar fat steam distilled for four hours, or until a distillate volume of about 900 milliliters was obtained. The distillate was divided into five portions and extracted with 50 milliliter portions of diethyl ether. The ether extract was dried over anhydrous sodium sulfate and reduced to a volume of one milliliter under vacuum at a temperature of 40 degrees C. or less. Barrow fat was similarly distilled and the extracts from both fats analyzed by gas chromatography using a five foot, one-fourth inch copper column packed with polyethylene glycol on Celite and a ten foot, one-fourth inch copper column packed with diethylene glycol succinate on Chromasorb.

Examination of Contents of the Preputial Diverticulum of Boars

The contents of the preputial diverticulum of several boars were combined, filtered and the filtrate extracted with an equal quantity of ether. This extraction was repeated and the combined ether extracts were washed with distilled water. After evaporation of the ether, some of the residue was smelled before and after heating, and some analyzed by gas chromatography using a 100 foot capillary column coated with Apiezon "L".

Tests for the Presence of Ammonia, Carbonyls, and Sulfur Compounds in the Volatile Distillate From Boar Fat

Ammonia test paper was used to test for ammonia in the volatile stream coming from the distilling flask containing boar fat with connective tissue. The paper was prepared by mixing ten milliliters of 20 per cent silver nitrate solution with five drops of 40 per cent formalin and a few drops of dilute sodium hydroxide. This mixture was filtered and the filtrate immediately absorbed on strips of Whatman 1 filter paper. After drying, the paper was then ready for use. In the presence of ammonia the paper turns black.

Carbonyls distilled from intact and from rendered boar fat were detected by passing the volatile stream through a solution of 2 - 4 dinitrophenylhydrazine (2 g. per liter in 2 N HCl) and by mixing the contents of the dry ice trap with the reagent. A yellow precipitate indicated the presence of carbonyls.

Sulfur compound(s) were detected by passing the volatile stream through or mixing the dry ice trap contents with a saturated solution of basic lead acetate.

Gas Chromatography of the Unsaponifiable Matter from Boar and Barrow Fat

The process of saponification and recovery of the unsaponifiable matter was given in the first section of the experimental procedure. The unsaponifiable matter from boar and barrow fat was heat tested and analyzed by gas chromatography using a ten foot, one-fourth inch copper column packed with diethylene glycol succinate on Chromasorb or silicone SE - 30 on Chromasorb "W". Several compounds suspected of being present in unsaponifiable matter were obtained in pure form and subjected to gas chromatographic analysis. Their retention times were compared with those of peaks produced by similar analysis of the unsaponifiable matter.

Column Chromatography of Unsaponifiable Matter from Boar Fat

A 161 milligram sample of unsaponifiable matter obtained from boar fat was subjected to silicic acid chromatography employing the method of Hirsch and Ahrens (1958). The column was 253 millimeters long and 23 millimeters in inside diameter. It was packed to a depth of 150 millimeters with 325 mesh silicic acid. The bottom of the tube was fitted with a sintered glass disk. The type and volume of eluents were the same as those recommended by Hirsch and Ahrens (1958) for the stepwise elution of complex lipid mixtures.

Paper Chromatography of Unsaponifiable Matter and Preparation of Chemical Derivatives

Paper chromatograms of the intact unsaponifiable matter were prepared on sheets of Whatman 1 filter paper. The chromatographs were made in an attempt to separate any free alcohols and aldehydes and/or ketones. The solvent system for the alcohols was n - butanol, ethanol, and water (4:1:5) while hexane and chloroform (9:1) was the solvent system for the free aldehydes and ketones. The free carbonyls were chromatographed on paper sprayed with a dilute solution of sodium bisulfite.

Attempts to prepare chemical derivatives of the alcohols were made by reacting three aliquots of unsaponifiable matter with 3 - nitrophthalic anhydride, α naphthyl isocyanate, and benzoyl chloride. The carbonyls were converted to their 2 - 4 dinitrophenylhydrazone derivatives by reacting the unsaponifiable matter dissolved in ethanol with a saturated solution of 2 - 4 dinitrophenylhydrazine in two normal hydrochloric acid. Derivatives were prepared by the methods outlined by Shriner and Fuson (1948).

The 3 - nitrophthalates and 2 - 4 dinitrophenylhydrazones were chromatographed on paper. Isoamyl alcohol, ammonium hydroxide and water (30:15:5 V/V) were used as the solvent system for the former derivatives and ethyl ether and petroleum ether (60 - 90 $^{\circ}$C.) in proportions of 5:95 (V/V) for the latter. All paper chromatographic procedures employed were outlined by Block *et al.*, (1955) or Lederer and Lederer (1957).

RESULTS AND DISCUSSION

PRELIMINARY STUDIES

Production of Sex Odor by Heating Boar Fat

There are rather wide variations between individuals with respect to their ability to recognize sex odor. Similarly, inconsistences can be noted between individuals asked to describe the typical odor. Frequently, the odor of decomposed urine in the prepuce and/or "piggy" odors in general are erroneously classed as sexual odor. Heat is not necessary to produce these odors, although it may increase volatilization and thereby result in a greater concentration in the surrounding atmosphere.

In this study, when boar fat was heated in a skillet the characteristic sexual odor was produced quickly and profusely. The odor was produced more slowly when fat was heated with water in an Erlenmeyer flask, probably because of lower heat and less surface area exposed to the heat source.

Sex Odor Volatilization Temperature

Sexual odor in heated boar fat was not apparent until the temperature of the lard bath reached approximately 100 - 108 degrees C. This indicates that the responsible component(s) are either present as precursors or are not volatile until a temperature of about 100 degrees C. is reached. It was interesting to note that when boar fatty tissues were heated in a skillet, the odor continued to be produced even though the temperature was much higher than 108 degrees C. If a sample of boar fat was heated, cooled, and then exposed to heat again, the odor seemed as intense as that produced initially.

Intensity of Sex Odor in Various Boar Tissues and Organs

Sex odor was classed as strong, slight, or none with respect to its

intensity in boar tissues and organs. The results of this part of the study are given in Table 2.

Table 2 Incidence of sex odor in various boar tissues and organs

Organ or tissue	Odor classification		
	Strong	Slight	None
Parotid gland	x		
Fat from diaphragm	x		
Testes	x		
Penis	x		
Preputial diverticulum	x		
Lean from loin (devoid of visual fat)		x	
Cardiac muscle (devoid of visual fat)		x	
Kidney		x	
Spleen		x	
Cowpers glands		x	
Seminal vesicles		x	
Sperm cord and overlying muscle		x	
Diaphragm muscle (devoid of visual fat)		x	
Tongue		x	
Lean residue after water extraction		x	
Fat residue after water extraction		x	
Liver			x
Lung			x
Water extract of lean			x
Water extract of fat			x

The data outlined in the foregoing table indicate that sex odor is rather widely dispersed throughout the various tissues and organs of the boar. It should be kept in mind that a certain amount of fat was probably an integral part of all the samples examined. The sex odor component(s) apparently are not water soluble, since sex odor could not be detected in water extracts of lean and fatty tissue, either while cold or on exposure to heat. When the residues from the water extractions were heated, however, the odor could be detected. Thus, the components responsible for sex odor are not soluble in water.

Effects of Curing Boar Meat on Intensity of Sex Odor

When cured boar bellies known to possess sex odor were heat tested,

the odor was still evident. This was true immediately after curing as well as after a 90 and 120 day cooler storage period. The odor seemed to diminish slightly, if at all, during this period of time. There was no discernible difference between smoked and unsmoked bellies. Obviously, the heat applied during smoking (about 120° F.) was not sufficient to cause any appreciable decline in the apparent residual odor. The results of the curing study are not in agreement with the observations of Kunze (1936), who claimed that "pickling" of a cryptorchid boar caused disappearance of the odor in 21 days.

Presence of Sex Odor and its Solubility Properties

Sexual odor could not be detected, when the frost collected in the receiver of a freeze drying apparatus was heated. The only noticeable odor was ammonia like, and very faint. However, on heating the residual freeze dried lean, sex odor was evident. Moisture-free, fat-free lean which was rehydrated in distilled water and heated was also free of sexual odor. Only the "brothy" smell of cooked meat could be detected. The ether extract obtained from the freeze dried lean was present in only small amounts and was burned on heating. This precluded definite detection of the odor in this fraction. However, since sex odor could be detected in lean tissue before but not after ether extraction, this seemed to be sufficient proof of its presence in the fat.

Sex odor was not evident in any of the samples of barrow fat and lean used in this study. Tissues from barrows were used as controls and served to contrast the odors noted for boar tissues.

It was stated previously that the sex odor components were not soluble in water. They were, however, soluble in some organic laboratory solvents. In addition, it was noted that the solvents varied with respect

to their ability to separate fat from its connective tissue. The results of the solvent extraction study are given in Table 3.

Table 3 Solubility of fat and sex odor components in various organic solvents

Solvent	Fat yield	Sex odor
Acetone	fair	+
Carbon tetrachloride	good	-
Chloroform	good	+
Dioxane	fair	-
Ethyl ether	excellent	+
Petroleum ether	good	+
Ethanol	fair	-

- indicates absence of odor
+ indicates presence of odor

Since some of the solvents used had high boiling points, difficulty was encountered in removing them from the extracted fat. The fat - solvent mixture was not heated above 180 degrees F., as a higher temperature may have destroyed or driven off the odor component(s). Sex odor could not be smelled in fat rendered with carbon tetrachloride, dioxane, or ethanol. Carbon tetrachloride has a rather dominant odor, and it could have masked the sexual odor. Ethyl ether seemed to be the solvent of choice, since it was very effective in removal of the fat containing the sex odor from connective tissue and was easy to remove from the fat due to its low boiling point.

Nitrogen Content of Rendered Boar and Barrow Fat

When wet neutral litmus paper strips were held in the volatile stream ensuing from heated boar or barrow fat, a basic reaction was noted. The color change was more rapid and the intensity greater in the case of boar fat. This difference suggested that the odor component(s) might be amine in nature, since some of these compounds have characteristically offensive odors. It was also recognized that the fatty tissue from boars used in this study contained more connective tissue than the fat from the barrows. Connective tissue is primarily protein and one would expect a larger quantity of ammonia on heating. When solvent rendered boar and barrow fat were analyzed for nitrogen, 0.149 and 0.141 per cent were found in each, respectively. The amount of nitrogen present was very small, and the difference noted between the two fats probably was of little significance.

Effect of Injecting Live Boars with "Diquel" Animal Tranquilizer

Fat samples, removed from four live boars by a biopsy technique, were heated and sex odor was found to be present in all four. Fat samples obtained from the carcasses of these four boars still contained the odor when exposed to heat. It seemed as intense after tranquilizer administration as before. Obviously, injection of the drug into live boars had no apparent effect on sex odor suppression in the carcass. It was theorized that animals rendered tranquil with Diquel might possibly withhold production or liberation of the agent(s) responsible for sexual odor should such agent(s) be liberated during periods of excitement. It should be emphasized that the relationship between excitement and liberation of the odor is purely supposition.

ISOLATION AND COLLECTION OF SEX ODOR COMPONENTS

Low Temperature - High Vacuum Distillation of Boar Fat

Freeze drying of cubed boar fat yielded a white frosty solid and dry fatty tissue. The frost melted shortly after the freeze drying was stopped. Application of heat to this liquid did not give rise to any

olfactory response suggestive of sex odor. The only noticeable odor was that of ammonia, and this was very faint. When the dried fat - connective tissue residue was rehydrated and heated in distilled water the volatile sex odor component(s) were detected. The flask containing the fat sample was at room temperature throughout the course of distillation. Results seem to indicate that heat is necessary to produce the odor. Many compounds are odor free when intact, but give rise to characteristic aromas when exposed to heat. The possibility that sex odor is produced by such a mechanism cannot be ignored.

<u>Gas Chromatographic Analysis of Volatiles Obtained from Boar and Barrow Fat by High Temperature - Atmospheric Pressure Distillation</u>

Boar fat with adhering skin was heated in a flask by means of an electric mantle and produced a number of familiar odors, but none could be classed as typical of sexual odor. The fumes emerging from the flask were "eggy", "meaty", and ammonical in nature, somewhat resembling cooking bacon or rendering lard. It was postulated that the odor component(s) might be basic in nature and as such could be trapped in an acid medium. Using a flask containing hydrochloric acid as the trapping medium, odors similar to those above were noted. Heating the trap contents intensified the odors only slightly and, as far as could be determined, did not give rise to sex odor.

When the volatiles were trapped in a flask immersed in a dry ice - ethanol mixture, the odors obtained were very much like those described above. Sex odor could not be detected on heating the trap contents. Obviously, replacing the air cooled condenser with a short length of glass tubing and freezing out the volatiles did not produce the desired results. Perhaps other factors were involved, such as length of heating

period, the temperature of the fat, the presence of connective tissue, or changes in the nature of the odor due to its concentration in the trap, and were responsible for lack of detection of the odor. It was possible that the sex odor was in the trap, but in such a concentration that it could not be recognized.

Ethyl ether seemed to be a good solvent for the component(s) responsible for sex odor, and hence it was used in an attempt to trap the volatiles. Considerable water was distilled over into the ether trap. The water was removed with a separatory funnel since the Barber-Colman Model 20 gas chromatograph does not operate efficiently in the presence of water. Reduction of the volume of ether to one milliliter provided a sample suitable for analysis. Examination of this material by gas chromatography was unsuccessful. No peaks were obtained on the chromatographic chart other than the solvent peaks.

When boar fat with the adhering skin was heated in distilled water for two hours, a number of volatiles were produced. The distillation apparatus employed by Kramlich (1959) was found to be a suitable assembly for trapping these volatiles. Nitrogen gas was bubbled into the fat to minimize possible oxidation, which probably occurred when air was used. However, the odor is usually developed in a normal atmosphere and in all subsequent distillations a stream of air, rather than nitrogen, was bubbled through the fat. Gas chromatographic analysis of the volatiles obtained indicated a number of compounds were produced, but no difference between boar and barrow fat could be detected. Similar results were obtained when a larger amount of fat (2500 grams rather than 500 grams) was used and heat applied for a longer period of time. Direct heating with a gas flame resulted in obvious burning of the fatty tissues and

probably produced different volatile compounds, therefore, a lard bath was used to replace the flame. Although burning was prevented, there was still no apparent difference in the volatile constituents from boar or barrow fat.

Undoubtedly, skin and connective tissue will produce various volatile components when heated. Ammonia and sulfur compounds probably came from this source. Since sex odor could be smelled in rendered fat, it was assumed that presence of connective tissue was not essential to the production of the odor. Therefore, to eliminate volatiles which might arise from skin and other protein material, the skin and connective tissues were avoided by using extracted fat in subsequent studies. However, no differences in the volatile constituents were noted between extracted boar and barrow fat when analyzed by gas chromatography.

The wet ice trap used in the high temperature, atmospheric pressure distillation assembly was employed primarily to remove water vapor from the volatile stream. Other volatiles were also collected in this trap and they had an odor very reminiscent of cooked fat. The remainder of the volatiles were frozen out in the dry ice - ethanol trap. Although the wet ice trap effectively removed most of the moisture, it is quite likely that it would also condense out the component(s) responsible for sex odor, since they did not appear to volatilize until a temperature of 100 - 108 degrees C. was reached. The wet ice trap was eliminated when distilling connective tissue - free fat since it contained less moisture than intact fat.

Contents of the dry ice - ethanol trap were analyzed by gas chromatography using a five foot diethylene glycol succinate column. The column temperature was maintained at 126 degrees C. and a gas pressure of 20

pounds per square inch was used, giving a flow rate through the column of 100 milliliters per minute. Generally, eight peaks were obtained when the trap contents were analyzed, but variations between seven and 11 were not uncommon. The number of peaks were generally the same for both boar and barrow fat. The peaks were rather sharp but not too well separated. Retention times were short, the last peak usually emerging from the instrument after ten or 12 minutes. Essentially no differences in peak location were detected between boar and barrow fat. When minor differences did occur, in most cases they were not reproducible. Application of heat to the condenser used during collection of the volatiles to avoid premature condensation did not increase the number of peaks obtained. As before, no sex odor could be noted in the trap when heated. Thus, it can be assumed that if the sex odor components were present in the trap at all, they were in an unrecognizable form. Better separation of the peaks obtained from distilled boar fat was produced by increasing the column length from five to ten feet. Gas flow rate was reduced to 50 milliliters per minute. The decreased flow and greater column length increased the retention time to about 14 minutes. Several additional peaks were obtained but distinct differences between boar and barrow fat were not observed.

Up to this point, short heating periods had not been employed in the collection of volatiles from boar fat. Since the longer heating periods were unsuccessful, it was decided to attempt collection of the sex odor components by short time heating. Intact, skinless boar fat was used. Short time heating on an electric stove did not cause the fat to burn, hence the volatiles from this source were minimized. The distillate collected in the wet ice trap consisted mostly of water and had a characteristic cooked fat smell. A frosty condensate was collected in the dry ice - ethanol trap. The ether extract of the wet ice trap was analyzed by gas chromatography using a ten foot column packed with diethylene glycol succinate. The column temperature was maintained at 112 degrees C. and argon flow rate at 30 milliliters per minute. This analysis yielded only one peak other than that produced by the solvent. If other volatiles were present, they were not in sufficient concentration to be detected. Analysis of the dry ice - ethanol trap indicated that a number of volatile constituents were present, although the sex odor could not be detected on heating this trap. Initially only one or two peaks were obtained. Increasing the sensitivity of the gas chromatographic instrument yielded additional peaks. In general, about eight peaks were obtained from both boar and barrow fat, although inconsistences were noted between samples.

Peak height varied between samples, indicating that the amount of a particular volatile component also varied. This variation was not due to differences in volume of gas injected, as this was constant throughout the study. Concentration of the vapor could vary, however, and this may have been the reason for the variation in peak height.

On one occasion the content of the wet ice trap seemed to evolve a faint sex odor when heated. This was the first time that any sex odor was noted in a heated trap. By distilling 6150 grams of fat, a total aqueous distillate of 300 milliliters was obtained. The ether extract of this distillate was yellow when reduced to a volume of two-tenths of a milliliter. Analysis of the ether extract by gas chromatography, using 100 foot capillary columns packed with either dinonyl phthalate or Apiezon "L" was not successful. No peaks other than the solvent peak were obtained. Of the ten foot packed columns employed, mannitol on Chromasorb "W" was most suitable as far as separation was concerned and it was used in all future trials employing the short time heat distillation assembly. Typical chromatograms obtained using this column are shown in Figures I and II. The chromatograms shown are made to the same scale as they appeared originally. It can be seen that about 14 peaks were obtained from both boar and barrow fat. The large peaks noted at the beginning of the chromatograms were produced by the solvent. Since no reproducible differences were noted between the chromatograms of the volatiles, no attempt was made to identify any of the peaks.

Under the conditions employed in this study, the agent(s) responsible for sex odor in pork were not retained in recognizable form in any of the traps used. The fact that the odor was produced in the heated fat, but was not detected in the traps, indicated the possibility that the component(s) responsible for sex odor could have been altered or destroyed during heating of the fat or were condensed before reaching the trap. Some of the residues from the component(s) could have possibly been retained in the traps, but did not produce sex odor when heated.

Analysis of Volatiles Obtained by Direct Sampling Methods

The ionization detector found on the Barber-Colman instrument is very sensitive. An attempt was made to take advantage of this sensitivity by analyzing a sample taken directly above hot boar fat from which sex odor was emerging. Initially, a small sample of about one milliliter was analyzed using a 100 foot capillary Apiezon "L" column. Injection was made into the liquid sample inlet of the instrument. No peaks were obtained except for one produced by a momentary increase in pressure when the gas sample was injected. This injection caused a drift of the base line gradually upward and then slowly downward. This difficulty could have been due to moisture in the sample although no such condition was observed when a five milliliter sample was injected into the instrument equipped with

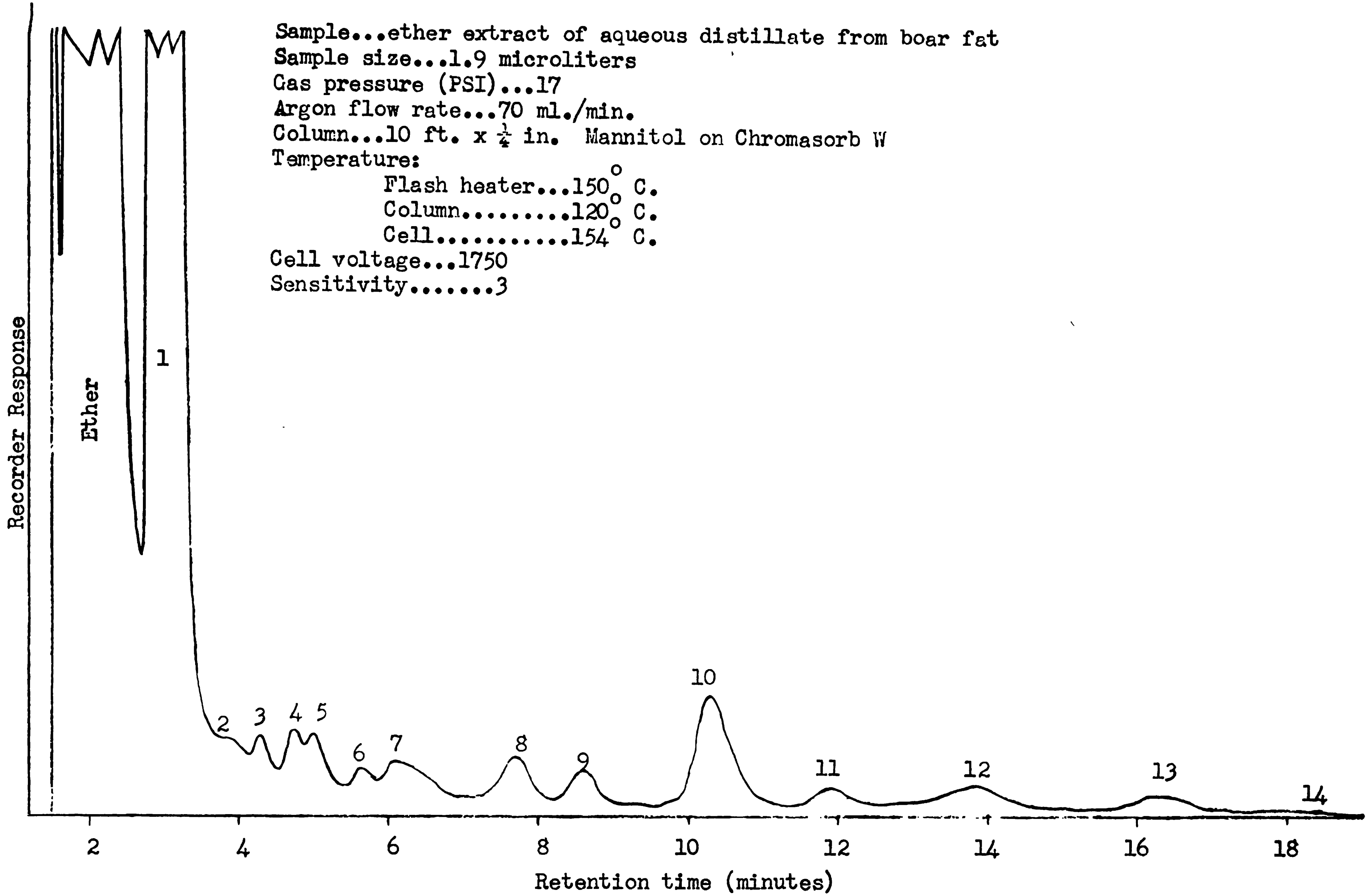

Figure I. Gas chromatogram of ether extract of aqueous distillate from boar fat

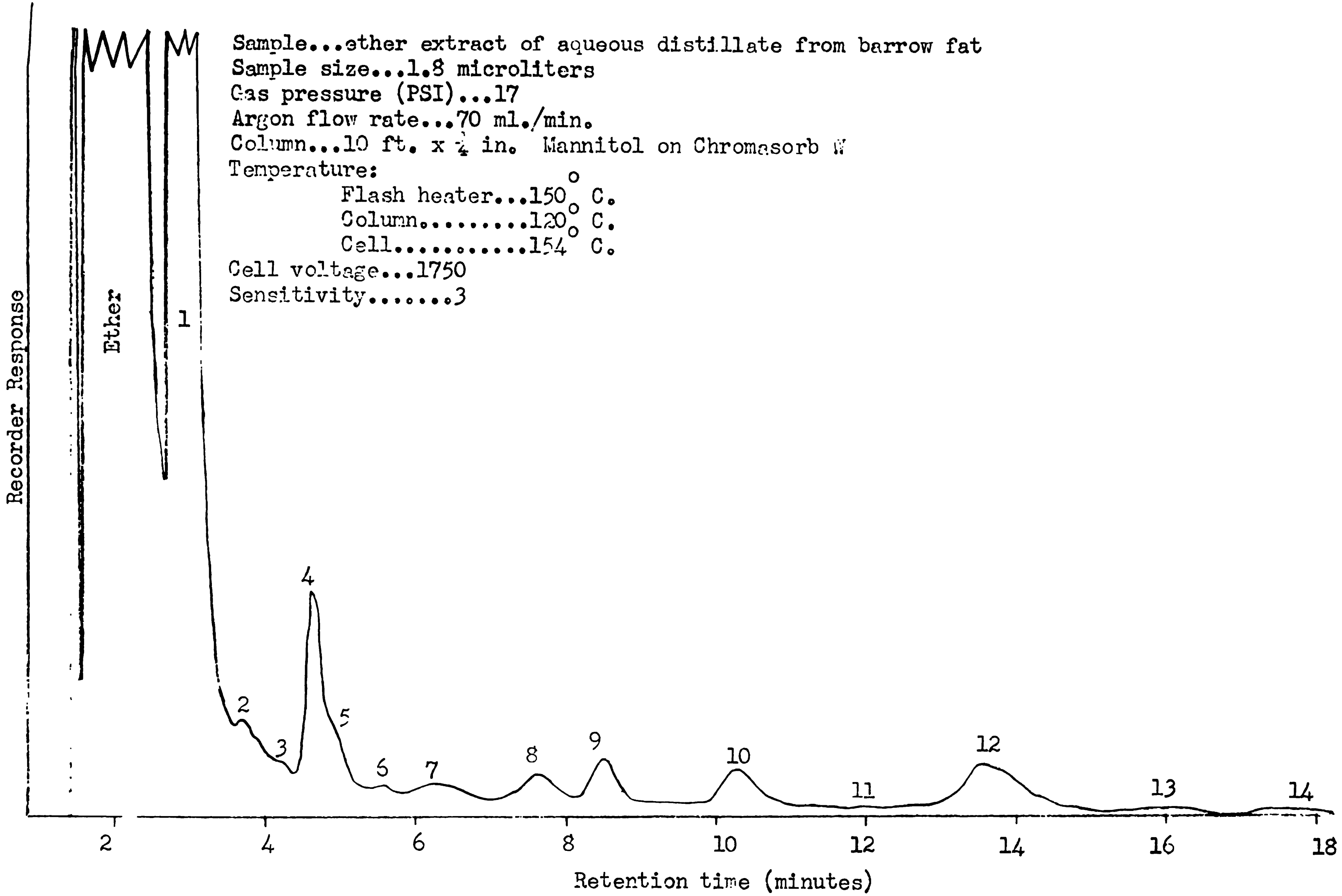

Figure II. Gas chromatogram of ether extract of aqueous distillate from barrow fat

a ten foot packed column. No peaks were obtained when the latter column was used. It is questionable whether capillary columns are capable of handling gas samples, since the volume of free space in columns of this type is so small. It would seem that if the sex odor component(s) were trapped at all by the direct sampling methods, they were not sufficiently concentrated for detection.

Analysis of Steam Distillate of Boar Fat

When 500 grams of boar fat were steam distilled for four hours, a distillate of about 900 milliliters was obtained. The ether extract of the distillate appeared to be colorless, but a yellow color was noted when the volume was reduced to one milliliter. Barrow fat was also light yellow when similarly treated. The aqueous distillate had a "sulfury" or "eggy" smell with overtones of ammonia. When heated, no indication of sex odor was noted. After ether extraction, the aqueous distillate still retained the sulfur-ammonia odor. Attempts to reconstitute sex odor by injecting a small volume of the ether extract into sex odor-free barrow fat and subsequently applying heat were unsuccessful. These results, coupled with an absence of sex odor in the steam distillate, indicated that sexual odor *per se*. could not be isolated and concentrated by the steam distillation method employed in this study.

Gas chromatographic analysis of the ether extract from the steam distillate did not reveal any differences between boar and barrow fat. In fact, only one peak other than the solvent peak was noted. This was true using both a five foot polyethylene glycol column and a ten foot diethylene glycol succinate column.

Analysis of the Preputial Glands and Preputial Diverticulum from Boars

Dutt *et al*., (1959) claimed that the preputial glands were the site of odor production. If this is true, heating of these glands should produce a more intense odor, unless it is distributed in the fatty tissues as rapidly as it is produced. On heating of the preputial glands or the fat surrounding the preputial diverticulum, the apparent sex odor was no more intense than that obtained from heating boar fat alone. Gas chromatographic analysis of an aqueous distillate produced from the preputial glands and the surrounding fatty tissues failed to show any difference from that observed when backfat from a boar was similarly analyzed.

Analysis of the Contents of the Preputial Diverticulum from Boars

The preputial diverticulum contains a liquid with a foul smelling odor. As previously mentioned, this odor is sometimes designated as "the" sexual odor, although it appears to be distinctly different. Since the present study had indicated that the sex odor component(s) was/were soluble in ether, this solvent was used to extract the contents of the preputial diverticulum. After evaporation of the ether, a urine-like smell was obvious. When the residue was heated, the same odor was noted except it was more intense. There was no indication that sex odor was present. When the extract was analyzed by gas chromatography, only the solvent peak was evident. Most of the material in the preputial diverticulum is probably water soluble, and one would expect a rather small quantity of ether soluble material. The urine smell was present in the ether extract, probably due to the fact that some of the odorous compounds in the urine were soluble in ether and were removed by the extraction process.

Presence of Ammonia, Carbonyls and Sulfur Compounds in the Volatile Distillate from Boar Fat

When ammonia test paper was held in the volatile stream emerging from heated boar fat, a black color was immediately noted on the paper. The color reaction indicated that ammonia was being evolved from the heated fat. The ammonia, no doubt, arose from the decomposition of some of the amino acids which make up the connective tissue of the fat. Tests for ammonia were negative when the vapors from connective tissue-free boar fat were checked.

It was suspected that a number of carbonyls were formed during the distillation procedure in which the fat was heated for five hours. When the contents of a dry ice - ethanol trap were reacted with a solution of 2 - 4 dinitrophenylhydrazine, a yellow precipitate was immediately obtained, indicating the presence of one or more carbonyls. Paper chromatography of the 2 - 4 dinitrophenylhydrazones, using petroleum ether (35 - 60° C.) and ethyl ether (95:5 V/V) as the solvent system, produced three spots. No attempt was made to identify the compounds responsible for the spots. When the volatile stream from heated, rendered, boar fat was passed through a solution of 2 - 4 dinitrophenylhydrazine before being trapped in the dry ice - ethanol trap, a yellow precipitate was produced. Again, no attempt was made to identify these hydrazones, but the presence of carbonyls was definitely indicated. Gas chromatographic analysis of the volatiles after removal of the carbonyls showed that several peaks had disappeared. Tests for the presence of sulfur compounds were conducted similarly by reacting the trap contents with lead acetate solution. The absence of a black precipitate indicated that sulfur compounds were not present. Gas chromatographic analysis of the volatiles after passage through the lead acetate solution indicated that no components had been removed. These results were not unexpected since the fat used in these determinations was free of connective tissue, and there were probably no sulfur containing compounds present.

<u>Presence of the Sex Odor Component(s) in the Unsaponifiable Fraction</u>

Initial saponification of boar and barrow fat produced an unsaponifiable fraction which was oily and appeared much like liquid fat. In addition, the amount of this fraction obtained was in excess of that expected. It was assumed that complete saponification had not been accomplished. Analysis of the fractions from the two types of fat by heat test and gas chromatography failed to show any distinct differences. Both fractions smelled rather sickly sweet, somewhat suggestive of esters. Heating of the soap by either wet or dry heat did not produce any olfactory response reminiscent of sexual odor. However, since complete saponification had apparently not been attained, the results of the above analyses were probably unreliable.

The saponification method used in this study was considered quite suitable since at no time during the procedure was it necessary to heat the saponification mixture. This gentle treatment prevented destruction of the apparently heat-labile sex odor component(s). Thus, subsequent saponification of boar and barrow fat was conducted so as to attain complete formation of soap from the fatty acids. The unsaponifiable matter obtained was yellow in color, and the yield amounted to one to two-tenths of one per cent of the fat. This fraction was stored in a refrigerator, samples being removed for use as required. Chilling caused the entire fraction to solidify and after a time patches of white crystals appeared dispersed throughout the solid mass. It was postulated that these crystals were cholesterol, and microscopic examination showed them to be identical in color and shape to known cholesterol crystals.

Unsaponifiable matter was tested for the presence of sex odor by placing a few small drops (about ten microliters) on the hot inlet port of the gas chromatograph. When the unsaponifiable matter was heated, a permeating, concentrated sex odor was immediately noted. A small amount of this fraction was injected into a piece of sex odor-free barrow fat. When this fat was heated in a skillet, the characteristic sex odor was detected. Heated unsaponifiable matter from barrow fat produced an odor which was somewhat sweetish, but it was not the characteristic sex odor and could not be classed as objectionable.

Analysis of the unsaponifiable fraction from boar and barrow fat using a ten foot diethylene glycol succinate column was not successful. On one occasion, several peaks were obtained but they could not be reproduced in subsequent trials. In addition, it was impossible to maintain a stable base line on the recorder. This was thought to be due to bleeding of the liquid phase caused by exposing the column to a temperature of 240 degrees C. The maximum temperature recommended for this type of column is 250 degrees C., but considerable column bleed probably occurs at a temperature of 240 degrees C.

The six foot, silicone SE-30 column, which can operate at temperatures up to 300 degrees C., indicated a number of compounds were present in the unsaponifiable fraction. However, essentially no differences could be noted between the two fats even though the sex odor was definitely present in the unsaponifiable matter from boar fat. A few minor differences were noted at times, but no real and consistent difference seemed to exist. These results were rather surprising. Perhaps no peaks were obtained because the heat of the column chamber destroyed the sex odor components and produced fragments which could not be observed on the chromatogram. Several chromatograms were run at 110 degrees C.; however, only a few peaks were produced at this temperature and those obtained were

similar for both types of fat. At the lower temperature poor resolution was obtained, apparently, due to the presence of the high boiling components which would mask other compounds.

Typical chromatograms using a 250 degree column temperature are shown in Figures III and IV. All peaks shown are represented as actual size except peak number 13. The width of this peak was reduced to one-half its original size. The component responsible for this peak seemed to constitute the major portion of the unsaponifiable fraction. This was assumed to be cholesterol since this compounds is known to be a component of the unsaponifiable fraction of fats. Analysis of pure cholesterol by gas chromatography produced a peak which compared in shape and retention time to the unknown peak observed in the unsaponifiable matter (Figure V). Further evidence for the presence of cholesterol was shown by the fact that a positive test for Δ - 5 sterols was obtained, when the white crystals from the unsaponifiable matter were dissolved in chloroform and reacted with acetic anhydride and concentrated sulfuric acid (Liebermann - Burchard reaction). The positive test was shown by the color of the reaction mixture, which varied from red to purple to bluish green.

Cholesterol is a known component of the unsaponifiable matter from both boar and barrow fat. Hence, it was not expected to be one of the agents responsible for sex odor in boars. When cholesterol was heated, the undesirable sex odor was not observed.

Squalene is also known to be a component of the unsaponifiable fraction of some fats. Analysis of a sample of squalene by gas chromatography (Figure VI) indicated that this compound had a retention time which compared to that of peak number 12 (Figures III and IV) present in the chromatograms of the unsaponifiable matter from both boar and barrow fat. No

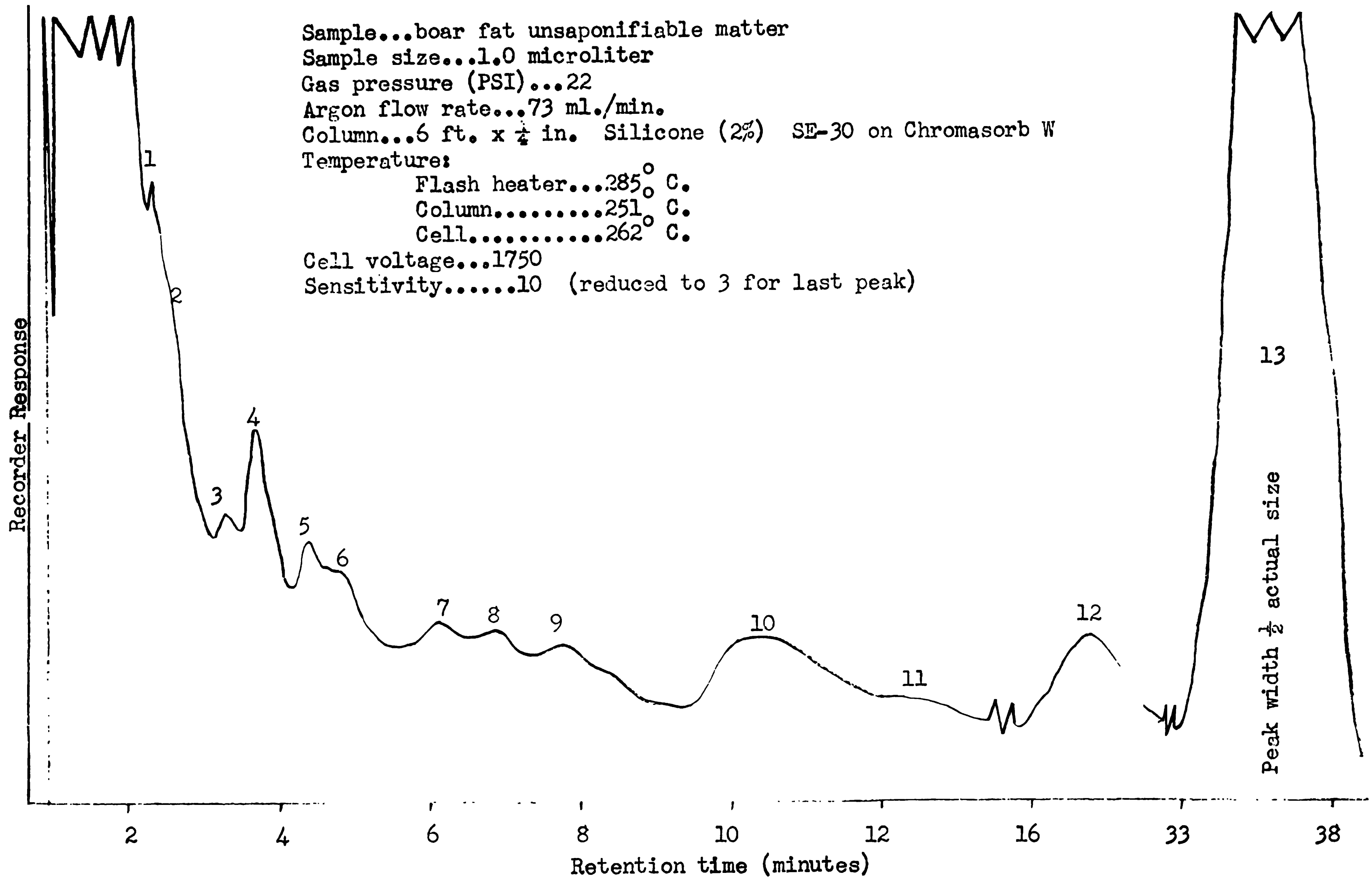

Figure III. Gas chromatogram of unsaponifiable matter from boar fat

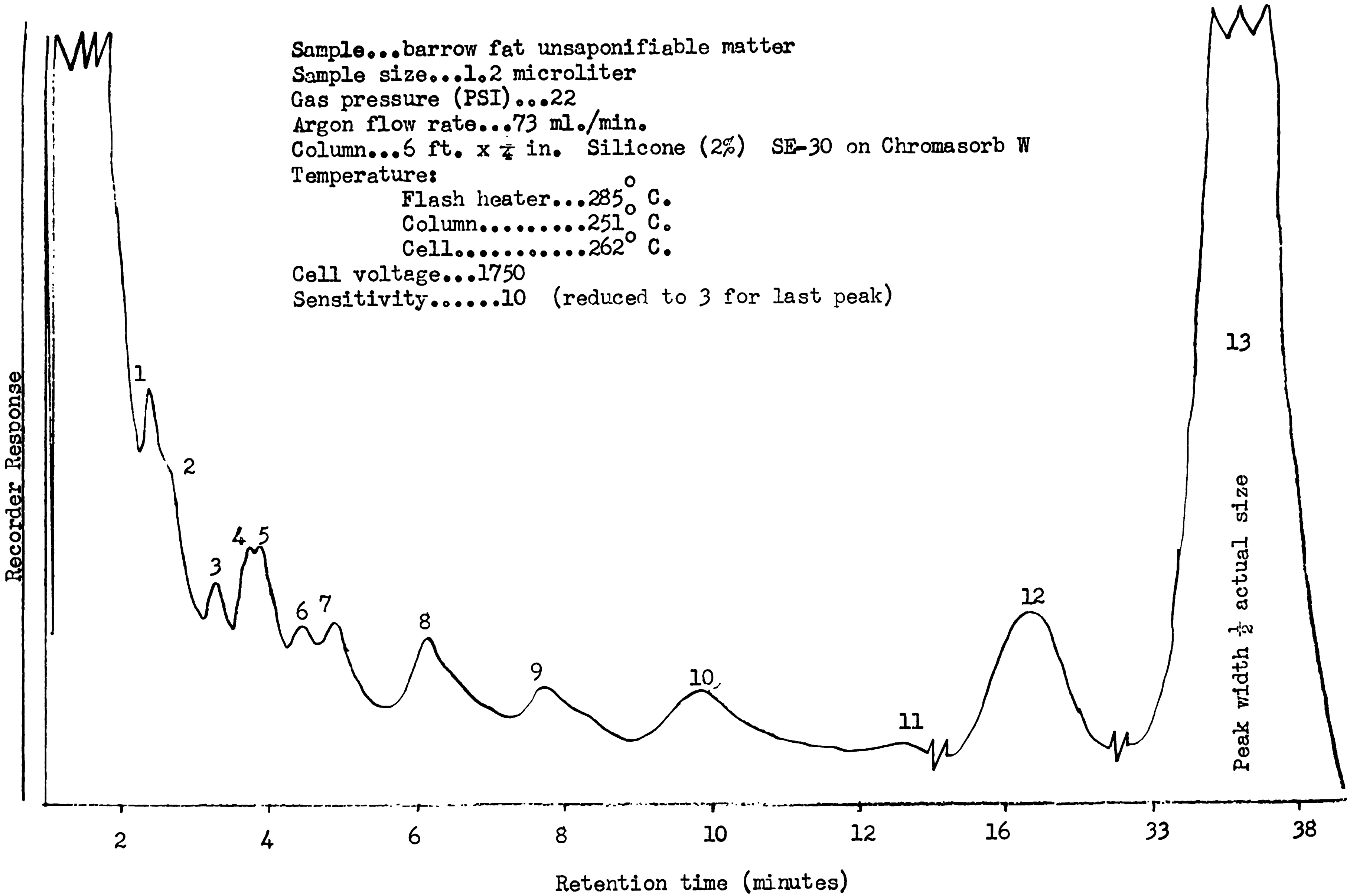

Figure IV. Gas chromatogram of unsaponifiable matter from barrow fat

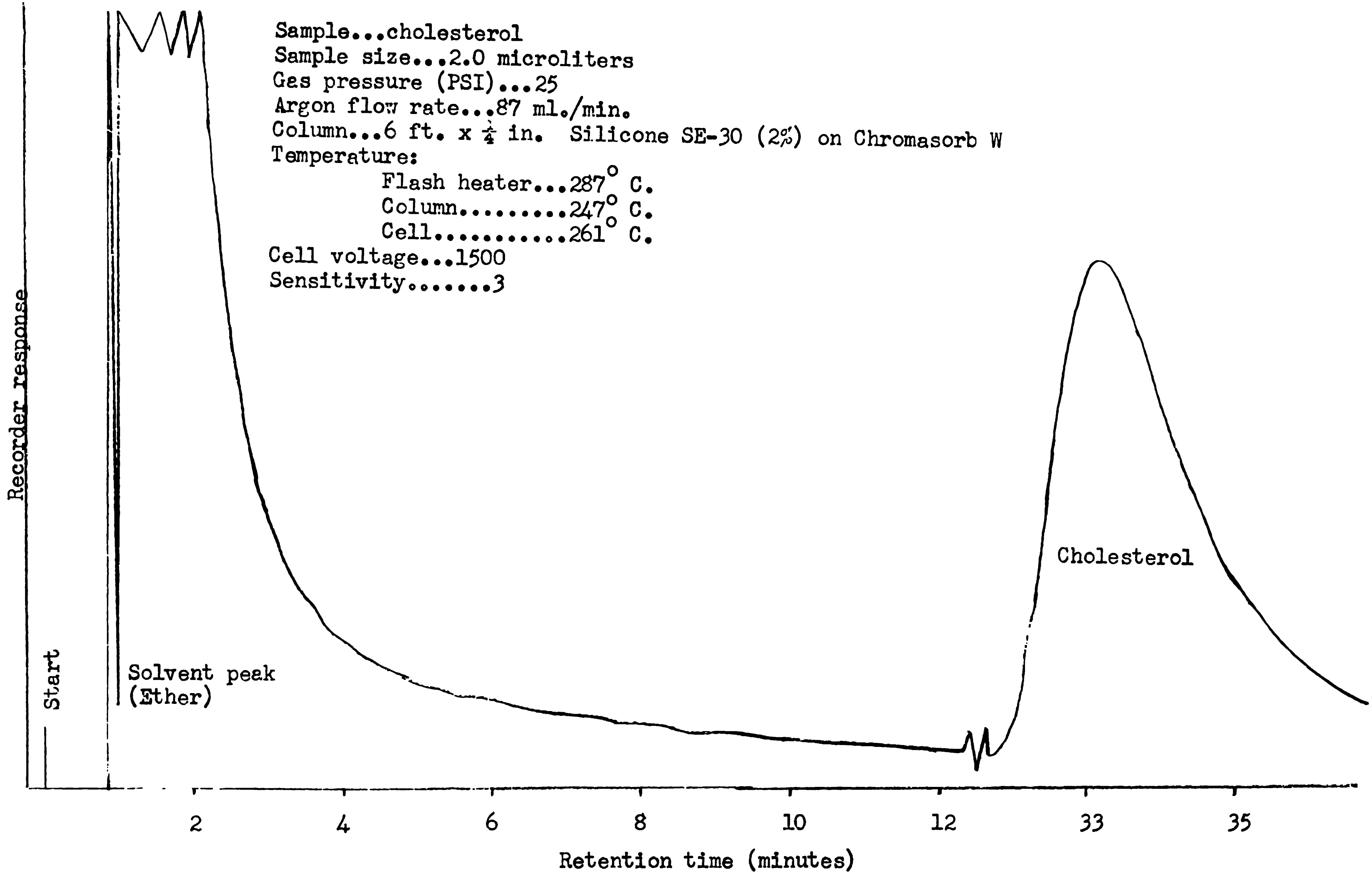

Figure V. Gas chromatogram of cholesterol in ether.

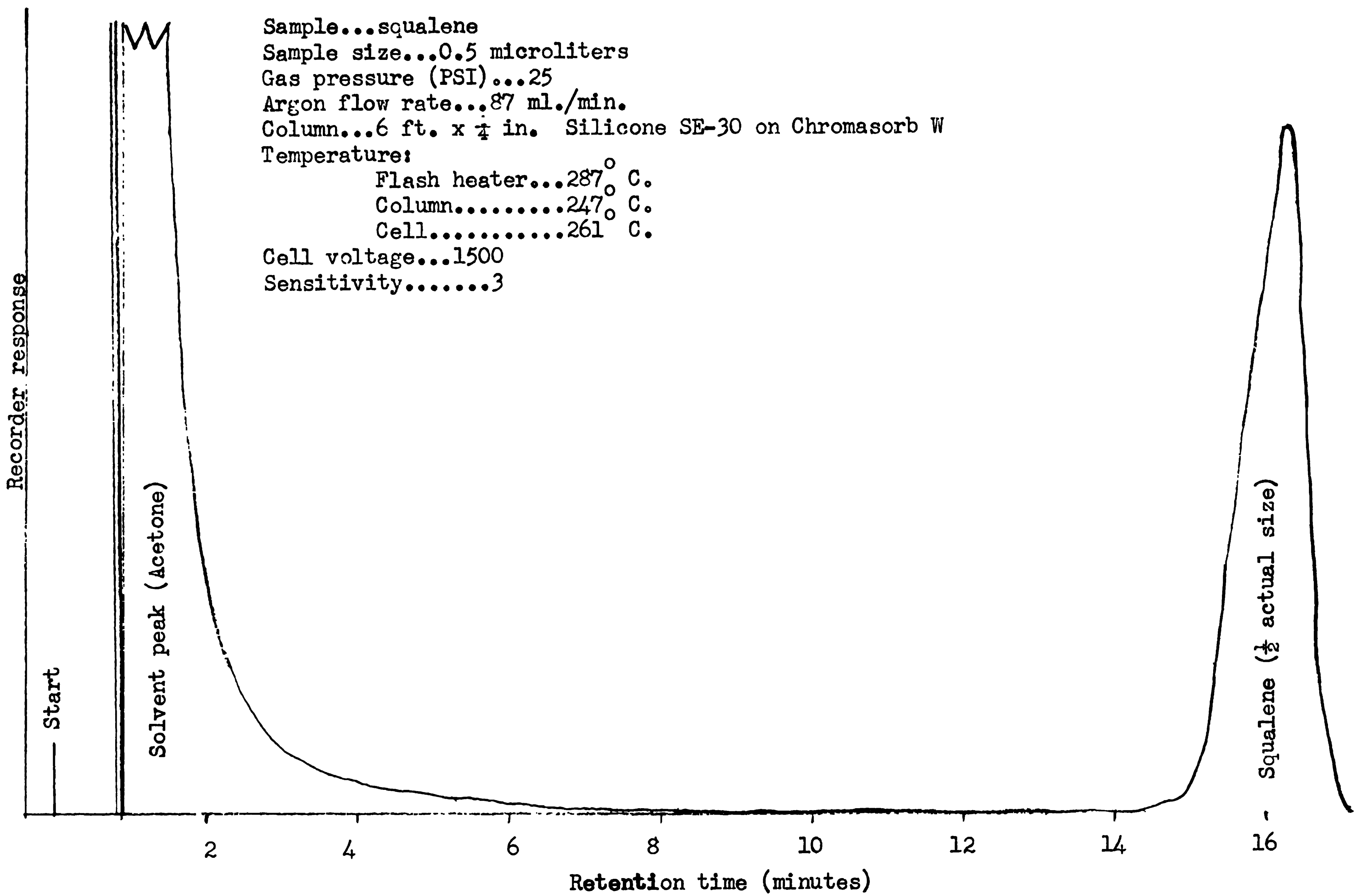

Figure VI. Gas chromatogram of squalene in acetone.

attempts were made to identify squalene by other qualitative means. Heating the known compound produced a rather odd, sweet odor, but it in no way resembled sex odor. Thus, of the compounds present in the unsaponifiable fraction, it seems justifiable to eliminate cholesterol and squalene _per se_, as being responsible for sex odor. It is still possible, however, that they may contribute to the development of sex odor by combination with other component(s) present in this fraction. Since squalene and cholesterol were present in both boar and barrow fat, it seems unlikely that they are responsible for sex odor.

Fractions Obtained by Column Chromatography of Boar Fat Unsaponifiable Matter

Hirsch and Ahrens (1958) list eight eluents varying in polarity from one per cent ethyl ether in petroleum ether to absolute methanol. When the unsaponifiable matter from boar fat was separated on a silicic acid column, three fractions were obtained. The first fraction was eluted with 200 milliliters of 25 per cent ethyl ether in petroleum ether (step 6). It was yellow in color and was present in a very small amount. Pure ethyl ether (step 7) eluted the largest fraction of the three. This fraction also was yellow in color and amounted to about 85-90 per cent of the charge placed on the column. On using pure methanol as the eluent (step 8), a small amount of yellowish brown liquid was obtained. When the three fractions were exposed to a heat test, it was found that the fraction eluted with ethyl ether contained the sex odor component(s). The fraction eluted in step 6 had a trace of the odor but it was absent in the material eluted in step 8.

Gas chromatographic (silicone SE-30 column) analysis of the three fractions did little to increase our knowledge of the problem. A few peaks were noted from the material eluted with 25 per cent ethyl ether

in petroleum ether and with absolute methanol (steps 6 and 8). The fraction obtained by elution with ether (step 7) appeared to be very similar to the unfractionated unsaponifiable matter. Thus, the separation of unsaponifiable matter was not complete when undertaken with the eluents employed. It is possible that a different series of eluents could be used to achieve a more efficient separation of the unsaponifiable fraction into its components.

Paper Chromatography and Chemical Derivatives of Unsaponifiable Matter

Paper chromatography of the intact unsaponifiable matter yielded one large yellow spot with a Rf of 0.94 when using a solvent system recommended for free alcohols. Two dimentional paper chromatography of this spot (Rf 0.93) failed to show that other spots were present. Paper chromatography of the 3-nitrophthalates of the alcohols produced one spot, which moved only a short distance from the base line and had a Rf value of 0.012. The crude derivatives of the unsaponifiable matter obtained with 3-nitrophthalic anhydride and αnaphthyl isocyanate could not be purified sufficiently to give a sharp melting point. Therefore, the presence of alcohols could not be verified by melting point determination. It seems that the occurrence of at least one alcohol in the unsaponifiable matter is a distinct possibility.

Similar chromatography of unsaponifiable matter to check for the presence of free-carbonyls failed to produce a discernible spot. However, a precipitate was obtained when the unsaponifiable matter was reacted with a solution of 2 - 4 dinitrophenylhydrazine. Paper chromatography of the hydrazone(s) formed from the carbonyl(s) produced a bright yellow spot with a Rf value of 0.94. The melting point of this derivative could not be determined due to an insufficient quantity of material. It appears that there may be one or more carbonyl(s) in the unsaponifiable matter.

SUMMARY AND CONCLUSIONS

Preliminary studies were undertaken to determine the location and solubility properties of sex odor in pork and to determine if an injection of animal tranquilizer into live boars would have any effect on suppression of sex odor in the carcasses. From these studies, the following conclusions were drawn:

1. Sex odor was produced when fat, lean and most organs from a boar were heated in a skillet or in boiling water. Sex odor was undetectable in cold tissues and, it was found that a temperature of 100 to 108 degrees C. was required for detection. This indicated that heat was necessary to volatilize the responsible component(s), or else it was essential for the formation of the compound(s) from precursor(s).

2. Water extracts of lean and fat were devoid of sexual odor. This indicated that the odor was not water soluble. However, it was found to be soluble in ether and several other organic solvents.

3. Moisture obtained from freeze dried lean tissue from a boar, as well as rehydrated, moisture-free, fat-free lean did not produce sexual odor on exposure to heat. The odor was noted, however, in dried lean tissue in which the fat was present. Thus, the agent(s) responsible for sex odor in pork are lipophilic and are not present in the fat-free lean.

4. Curing boar bellies by accepted curing methods did not cause suppression or disappearance of sexual odor. The odor appeared to be as evident after 120 days of cooler storage as it was initially.

5. The nitrogen content of solvent rendered boar and barrow fat was very low and the difference between the two fats negligible. Thus, sex odor probably is not due to nitrogenous compounds.

6. Injection of Diquel animal tranquilizer into live boars did

not seem to have any obvious effect on sex odor suppression in the carcass.

The second phase of this study dealt with the employment of various distillation and fractionation techniques in an effort to isolate and identify the agent(s) responsible for sex odor in pork. The procedures used included low temperature - high vacuum distillation, high temperature - atmospheric pressure distillation and steam distillation. Volatiles produced by the various distillation methods were collected in traps immersed in dry ice - ethanol or in ice water. The trap contents were analyzed by the heat test and gas chromatography. Fractionation procedures included saponification of boar and barrow fat with subsequent analysis of the unsaponifiable fraction by heat test, and by gas, paper, and column chromatography.

The following observations were made from this portion of the study:

1. Heating the contents of the traps failed to produce sexual odor. It was possible that the agent(s) responsible for sexual odor were destroyed by the heat applied to the fat or that they were condensed in the distillation assembly before reaching the trap. Thus, the various distillation methods employed for trapping the sex odor component(s) were not considered satisfactory.

2. Gas chromatograms of the contents from the dry ice - ethanol trap and ether extracts of the contents from the wet ice trap failed to show any consistent and reproducible differences between volatiles from boar and barrow fat.

3. Although cold saponification of boar and barrow fat yielded only a small quantity of unsaponifiable matter, the material obtained from boar fat produced a concentrated, permeating sexual odor on exposure to the

heat test. Thus, the agent(s) responsible for sex odor in pork are located in the unsaponifiable fraction of fat.

4. Gas chromatographic analysis of the unsaponifiable portion obtained from boar and barrow fat failed to show any distinct and reproducible differences between the two fractions even though the boar fraction was known to contain the sex odor.

5. Cholesterol and squalene were found to be present in the unsaponifiable matter from both boar and barrow fat, however, when these compounds were heated, sex odor was not produced. Thus, of the compounds present in the unsaponifiable fraction it seems justifiable to eliminate cholesterol and squalene *per se*. as being responsible for sex odor.

6. Paper chromatography of intact unsaponifiable matter, as well as of chemical derivatives prepared from this fraction, indicated that one or more alcohols or carbonyls may be present.

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