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A COMPARATIVE GAS CHROMATOGRAPHIC STUDY
OF VENISON AND BEEF FLAVORS

by

K. Prakash Reddy

A thesis submitted in partial fulfillment
of the requirement for the degree

of

MASTER OF SCIENCE

in

Food Science and Technology DEPARTMENT OF NUTRITION
& FOOD SCIENCES
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INTRODUCTION

Venison is an important food item in the diet of the people of the United States. Deer is hunted both as sport and for food. Annually, nearly 180,000 hunters go afield in Utah in search of deer. Over 130,000 animals are killed which amounts to an annual harvest of over 15,000,000 pounds of dressed venison. This contributes about 10 1/2 million dollars to the economy of the state from licenses, lodgings, transportation, and other expenses of hunting.

Venison as a whole has characteristic flavor and/or odor which is acceptable if mild, but some venison has an objectionable strong flavor. Because of the practical importance of the subject of venison flavor, this investigation has been undertaken to study the substances suspected of contributing to the flavor.

In this thesis is presented a comparative gas chromatographic study of the volatile substances which are assumed to include the characteristic flavor of venison and beef muscle tissues. Solubility properties of the volatile components were determined by ether-extraction and water-extraction.

This is one phase of the over-all project on investigations of venison quality and palatability being made under Federal aid project W-97-R through the Utah Department of Fish and Game. Previous work on the influence of field care

and aging of carcass, age of animal, season of harvest, quality of range, freezer storage, and addition of fat to ground venison, have been reported by Smith (1961), Bardwell (1963), and Bardwell et al. (1964).

Ever since civilized man inhabited the earth, flavor has been and still is one of the most important attributes of food. The word flavor conjures many ideas in the minds of many individuals. In order to have everyone agree on the meaning of the word, a definition of terms is desirable. A favorite definition of Kurtz (1959) is that flavor is a complex of sensations resulting from the stimulation of the senses of odor, taste, feel and sometimes vision and audition. Dimick and Corse (1956) define flavor as that which involves taste and odor. Taste is that which we recognize with our tongue. Odor depends on volatile materials which are detected by our olfactory centers. To the chemist, however, the combined senses of odor and taste are of importance in his work on flavor.

The actual chemistry of flavor is a relatively new area of fundamental research. Identifying the components responsible for the flavor and/or odor in foods is one of the most difficult problems a Food Technologist has to face.

Characteristic flavor (odors and taste) of all biological materials is due to chemical reactions and chemical compounds produced by the organism as a result of its normal metabolic processes (Hewitt et al., 1956). Presumably such flavors have been formed from other chemical substances, which can be

called flavor precursors.

It has been further shown by Hewitt et al. (1956) that these chemical changes are brought about by the catalytic effect of enzymes, the naturally occurring proteinaceous materials found in the organisms. Enzymes are often highly specific for bringing about the chemical changes in each step of the complicated process whereby the flavor precursor is built up and then converted into the flavor itself.

REVIEW OF LITERATURE

Little scientific work has been done on determining the components which are responsible for the flavor and/or odor in venison meat. A survey of the available literature shows that other red meats have been studied extensively. Many of the different methods used in other meats could be applied to venison.

The series of papers gathered in this review of literature is a coordinated attempt to survey the field of meat flavors and to outline some of its more important problems.

One of the earliest investigations carried out on meat flavors was reported by Barbella et al. in 1936, who observed that the feeding regimen of lambs influenced the flavor. According to Howe and Barbella (1937), there are certain inherent flavors in the fat of meat that are characteristic of species, which become evident when the fat is heated. Age of the animal also has an important influence on flavor of the meat.

Crocker (1948) stated that the typical meaty flavors were present in meat fibers rather than in the expressible fluid of cooked meat. More recently Kramlick and Pearson (1958) found that fluids expressed from raw meat developed a more concentrated flavor upon cooking. Barylko-Pikielna (1957) reported that taste panel^o evaluation indicated that

the typical flavor of roast beef was present in the water-insoluble residue, but that the water-soluble fraction of roast beef had an intense but atypical beef flavor.

The recent classic work of Wood and Bender (1957) should be mentioned as typical of the type of investigations common with meat flavors. They isolated and identified more than 30 volatile and non-volatile compounds from commercial ox muscle extract. Recently, Hornstein and his associates (1960) identified acetone, acetaldehyde, formaldehyde, hydrogen sulfide, ammonia, and methylamine from the volatile fraction of raw ground beef heated in vacuum. These authors also indicated that the flavor precursors were extractable with water. Kramlich and Pearson (1960) reported the presence of carbon dioxide, acetone, acetaldehyde, methyl mercaptan, and possibly methyl sulfide in the volatile fraction from cooked beef.

Batzen et al. (1960) made attempts to isolate and characterize some of the constituents of beef muscle tissue which evolve the distinctive odor and flavor associated with cooked beef. Beef muscle tissue was fractionated into a number of water-soluble and water-insoluble fractions. They found that dialysis of the water extract of uncooked ground beef gives a diffusate which, after lyophilization, is capable of producing an odor similar to that of broiled steak when heated with fat, and a beef broth odor and flavor when boiled in water. Recently Batzen et al. (1962) found that some of the precursors of meat (beef) flavor are a

relatively simple mixture of glucose, inosinic acid, and a glycoprotein. Therefore, they conclude that only certain of the amino acids in the glycoprotein are necessary precursors of meat flavor.

Hornstein and Crowe (1960) pointed out that the odor responses, and the chemical compounds isolated from the volatile pyrolysis products of lyophilized cold water extracts of lean beef and lean pork were basically similar as shown by the gas chromatograph. They suspect that the flavor difference that exists in pork and beef may have their origin in the fat portions of these meats. Hornstein et al. (1961) found that the cold-water extracts of lean beef and lean pork contain desirable meat-flavor precursors, whereas the lipids, especially phospholipids contribute undesirable meaty flavors. This is true according to Younathan and Watts (1960); as they suggested, that the phospholipids play a major role in accelerating flavor deterioration in cooked meats.

More recently Hornstein and Crowe (1963) working with lamb, showed that the characteristic aroma of heated lamb is obtained from the fat. The lean meat portions contribute a basic meaty flavor similar to that obtained from lean beef and lean pork.

According to Hornstein and Sulzbacher (1962), the lean portions of meat from beef, pork, and lamb contribute a similar basic flavor. They have pointed out that the flavor differences between meats reside in the fat portions. Free

fatty acids and volatile carbonyls in beef, pork, and lamb show qualitative differences that may influence flavor. The gas chromatographic patterns for the volatile obtained from animal fat are generally more complex than those obtained from lyophilized extract of lean meat.

Dutt et al. (1959) concluded that the preputial glands produced a fat diffusible material responsible for sex odor in boar carcasses. Similarly, in deer the cutaneous glands possess a characteristic strong odor which might influence the venison flavor.

MATERIALS AND METHODS

Selection and Classification of Samples

The left and right legs from 6 (4 of which were classified as mild venison and 2 as strong venison) deer which had been aged for seven days at 34 to 36 F were obtained through the Utah State Department of Fish and Game (Table 1). Cuts of these legs were roasted. A panel of judges scored the meat for the flavor and odor and classified it according to the amount of venison flavor present as strong venison or mild flavored venison. The cuts of meat from animals of strong venison and of mild venison were selected and stored under commercial locker conditions in the frozen state. Beef (round steaks) from four animals were obtained from a local grocery store and stored along with the venison samples until analysis was made.

Cutaneous glands of deer, namely, the tarsal and metatarsal, were also obtained from freshly killed animals and stored in the frozen state.

Samples were prepared for analysis on the gas chromatograph following the method with slight modifications of Hornstein et al., 1960. As needed, 120 grams of ^{copy} thawed and fat-free muscle was ground in an electric grinder in a room maintained at 32 F; Eighty grams of ^{then} ~~this~~ was blended with 120 ml. of cold distilled water, ^{omit} in an electric Waring blender. ^{omit} (also maintained at 32 F). ^{omit} The slurry was allowed to stand ^{omit} ~~at 32 F~~ ^{Insert I}

Table 1. Age, sex, and location of harvest area of deer for flavor study

Animal number	Cuts from the animal	Age of the animal	Sex of the animal	Location	Date of killing	Panel evaluation
3	Leg roast	Yearling	Male	Tony Grove Cache County	Sept. 7, 1962	Good
8	Leg roast	Faun	Female	Pintura Washington County	Nov. 11, 1962	Good
9	Leg roast	Mature	Female	Virgin Washington County	Nov. 17, 1962	Good
11	Leg roast	2 1/2 year	Male	Rich County	Dec. 11, 1962	Good
A	Leg roast	Mature	Female	Logan Canyon Cache County	Nov. 4, 1963	Strong
C	Shoulder	--	--	Nevada	1962	Strong

overnight of 32 F. blended again for 1 minute, and centrifuged at 4,000 rpm for 20 minutes in a refrigerated centrifuge kept at 28 F. The supernatant liquid was decanted, mixed with 1 per cent w/v of filter-aid, and then filtered under vacuum through a Buchner funnel. This filtrate was freeze-dried. *To here*

Another sample of 30 ^{gm} grams of ground meat was blended ~~as above~~ with 100 ml. of ^{omit} (cold distilled) water. The whole meat slurry ^{omit} was then ^{and} freeze-dried. *Insert II*

Same # Each sub-cutaneous gland was thawed ^{shared of} and the hair ^{omit} shaved from it. ^{omit} This was sliced, ^{omit} [into small pieces], blended with 50 ml. of ^{omit} (cold distilled) water, and ^{omit} (then) freeze-dried.

All the freeze-dried samples were weighed and ^{omit} kept refrigerated, ^{omit} in air tight containers until further use. *To here*

Determination of solubility properties
and location of flavor components in
muscle tissue

Begin One gram samples of lyophilized whole ^{omit} meat were weighed, *Insert III* [into clean extraction thimbles. Each sample was] extracted with 100 ml of ether for 5 hours, [using the Soxhlet extraction apparatus. Then,] the ether extract was evaporated ^{to dryness} on a steam bath [and was further ^{omit} evaporated to dryness, ^{and} in a vacuum dessicator.]

The lipid-free portion ^{omit} [of the samples (after ether extraction)] was water extracted with 50 ml. of (distilled) water and freeze-dried. The two extracts (ether and water extracts) were judged by sensory evaluations to determine the solubility of the flavor components. *To here*

Gas Chromatographic Operating Conditions

A simple and improved collection system of the volatiles and the gas chromatographic analysis, as reported by Hornstein and Crowe (1962), was followed with slight modifications.

The gas chromatograph was an Aerograph Model 600 C equipped with a flame ionization detector and incorporating linear temperature programming. The insulated column heating chamber had a 6 inch inner diameter and was 4 1/2 inches in height. The columns described were fitted to these dimensions.

The column

Columns (of 0.025 inches inner diameter) were 7.5 feet long, packed with 20 per cent castorwax on 30- to 60-mesh acid washed chromosorb W. Each column consists of two sections, joined by a Swagelock union. A 5.5 foot section was coiled into a helix, 2 1/4 inches inner diameter, and a 2 foot section coiled into a helix with a 1 inch inner diameter. The ends of this small coil were so arranged that the coil could be conveniently suspended in a small Dewar flask.

Collection of volatile compounds

A weighed amount of freeze-dried sample was placed in a clean, dry 250 ml Erlenmeyer flask. The flask was heated on a paraffin oil bath at 100 C for 20 minutes. The collection coil was kept immersed in a Dewar flask containing liquid nitrogen (Figure 1). Flow rate of the sweep gas, water pumped nitrogen, was adjusted to 15 ml per minute.

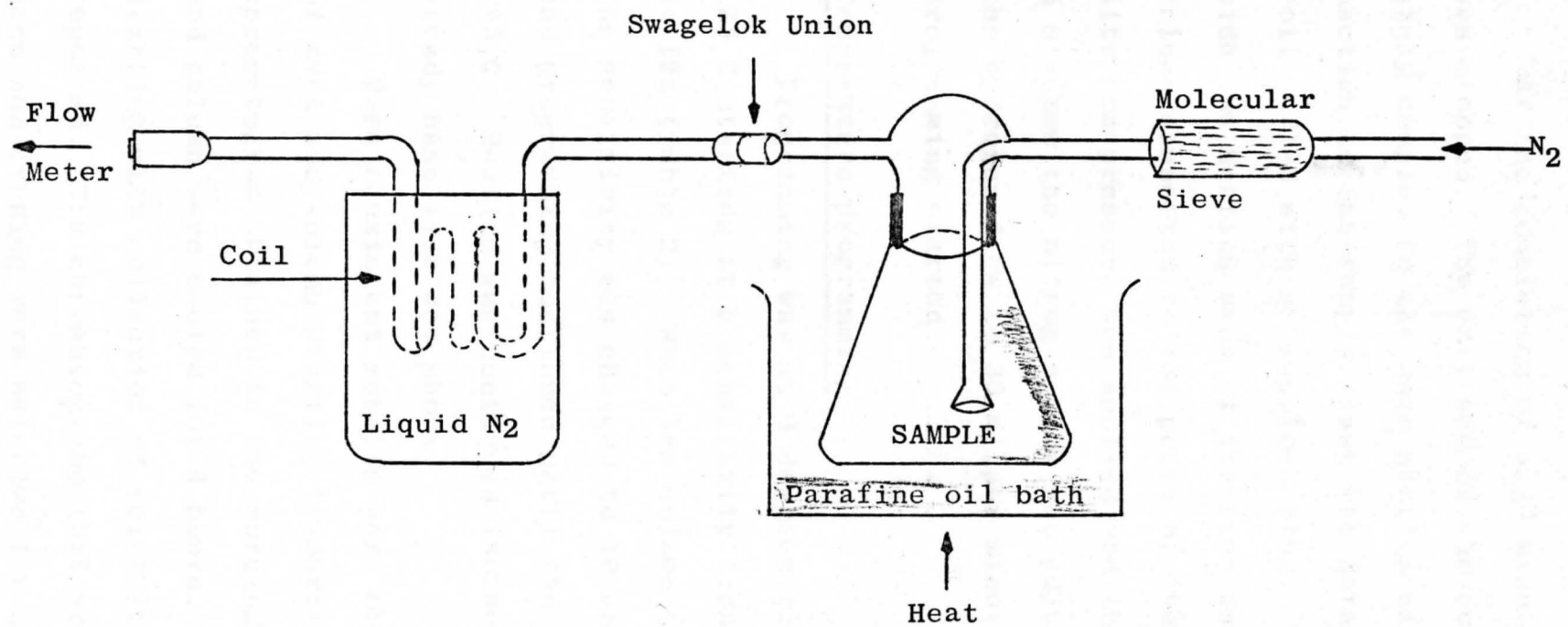


Figure 1. Assembly for collection of volatiles

At the completion of a 20 minute run, the nitrogen flow was stopped. The exit end of the collection coil was immediately coupled to the main section of the column. The connection to the sample flask was detached and this end of the coil sealed with a Swagelock plug. The plug was removed inside the heating area of the oven and ends of the composite column connected to the ports of the oven. A slight positive nitrogen pressure was applied and the flame ignited. After 5 minutes the nitrogen flow was adjusted to 12 ml per minute, the hydrogen flow to 30 ml per minute, and the temperature programming started.

Temperature programming

Programming was at 4 degrees rise per minute from 32 to 125 C, starting at a sensitivity (range) of 100 and attenuator at 128 (Table 2). When the column temperature reached 50 C, the sensitivity was changed to 10 and the attenuator to 4, and programming continued until the column temperature reached 125 C. Heating was continued isothermally at 125 C until a steady base line was shown.

More consistent results were obtained by careful control of oven and column starting temperature. Therefore, the chromatogram obtained in the morning was discarded. The oven and column were cooled for 4 hours. Then the whole procedure starting with collection of volatiles from a new sample was repeated. The chromatograms that were obtained in the afternoon and evening were selected for use.

Table 2. Gas chromatographic operating conditions used in the venison and beef flavor study

Sample size, grams	0.2
Column length, feet ^a	7.5
Column inner diameter, inch	0.025
Column material	20% Castorwax 30/60 Chromosorb W
Hydrogen flow rate, ml/min	30
Nitrogen flow rate, ml/min	12
Starting temperature, °C	32
Programming temperature, °C/min rise	4
Final temperature, °C	125
Starting sensitivity (range)	100
Starting attenuation	128
Sensitivity (range) at 50 C column temperature	10
Attenuation at 50 C column temperature	4

^aConstructed of stainless steel tubing

Calculation of results

The chromatograms were compared by two methods. Firstly, a comparison of the retention times (or retention volumes) of all the samples. Secondly, individual peak areas were calculated in square centimeters and these values compared. The areas were calculated by the method shown by Cremer and Müller (1951).

RESULTS AND DISCUSSION

Solubility Properties of Flavor Components

Lean muscle

The lean muscle when heated was found by sensory tests to have the odor of the venison or of the beef. This odor was detected in the lean portion to a much greater degree than in samples of heated fat. A water extract of the muscle when heated had the same odor and of a similar intensity to that obtained from the meat. Hence, the work reported in this thesis was directed to a study of the volatile components from the lean muscle. A water slurry of lean muscle and a water extract of the same meat were used for all tests on the venison and beef samples.

The distribution of flavor and their precursors between beef solids and juices have been studied by Crocker (1948) and more recently by Kramlich and Pearson (1958); their results indicated that flavor contributors were water soluble. Hornstein et al. (1960) stated that the flavor precursors of cooked beef are water soluble. Lyophilization of a water extract of beef yields a powder concentrate which on heating develops a flavor similar to that of cooked beef.

Ether extraction followed by water extraction

The sensory evaluation of the ether extracts and water extract of lyophilized whole muscle tissue of venison and of

beef is shown in Table 3. ^{start} Although ^{the sensory evaluations indicated that} the volatile components of venison and beef ^{in Experiment I} were much more pronounced in whole muscle tissue before ether extraction both the ether extract and the water extract (^{omit} showed that) each contained some of the volatile components. The results of the judges sensory evaluation ^{Insert III} indicated that the odor was slightly stronger in the ether extract or fat obtained from the lean muscle. However, a considerable portion of the volatile components was detectable in the water extract. ^{These data} (^{omit} The above) ^{omit} findings were further confirmed by gas chromatographic analysis. ^{To here}

Gas Chromatographic Analysis

Throughout the gas chromatographic analysis for all the samples, operating conditions were kept the same. During the preliminary trials one initial peak, having high concentration of odor, ran off the chart, primarily, due to high sensitivity (10) and low attenuation (4). In order to keep this peak within the chart, the sensitivity was lowered and attenuation increased to 100 and 128, respectively. At 50 C column temperature, the sensitivity and attenuation were changed to 10 and 4, respectively. This was maintained unaltered during the rest of the run.

Attempts were made to capture volatile compounds produced by heating a 0.2 gram of lyophilized sample, in a collection coil immersed in dry ice-acetone mixture. This did not trap any compound; whereas liquid nitrogen was effective in

Table 3. Sensory evaluation of ether soluble and water soluble portions of lyophilized whole muscle tissue of venison and beef

Animal number	Grams sample ether extracted	Ether solubility	Water solubility (lyophilized)
<u>Venison</u>			
A leg ^a	1.0090	Slightly strong	Mild
A leg ^a	1.0042	Slightly strong	Mild
C shoulder	1.0008	Mild	Mild
3 right leg	1.0013	Mild	Mild
3 left leg	1.0013	Mild	Mild
8 right leg	1.0007	Mild	Mild
8 left leg	1.0015	Mild	Mild
9 right leg	1.0006	Mild	Mild
9 left leg	1.0050	Mild	Mild
11 right leg	1.0105	Mild	Mild
11 left leg	1.0201	Mild	Mild
<u>Beef</u>			
Beef 1	1.0058	Mild	Very little
Beef 2	1.0021	Mild	Very little
Beef 3	1.0001	Mild	Very little
Beef 4	1.0012	Mild	Very little

^aLeg samples from the same animal; left and right sides were not labeled.

capturing the compounds. Hornstein and Crowe (1962) found that they had to use liquid nitrogen to trap the volatile compounds. Column material could also be a possible explanation for this difference. Craig et al. (1962) suggested that mannitol on chromosorb "W" proved to be the most suitable column packing material for separation of the ether extract of the aqueous distillate obtained in the dry ice trap. However, according to Hornstein and Crowe (1962), columns packed with 25 per cent castorwax on 30 to 60 mesh acid-washed chromosorb "W" was a better packing material to trap volatiles in a collection coil immersed in liquid nitrogen. Castorwax (20 per cent) on 30 to 60 mesh acid-washed chromosorb "W" was used for the columns for all gas chromatographic analysis of volatile compounds in this study.

^{copy} In most cases 7 peaks were obtained from both ^{venison and} beef and ^{Begin} venison. The individual peak areas were calculated by the ^{Insert} method of Cremer and Müller (1951). The chromatograms from the water soluble portion represented approximately 6 times more meat than those from the whole muscle tissue, (Table 4). Hence, peak areas of the two types of samples could not be compared directly. ^{To have} The 0.2 gram ^{of} dried water extract was from 80 grams of meat whereas the 0.2 gram of dried whole muscle was from 30 grams of meat.

Comparison of Peak Areas

The interpretation of whether the peaks of a chromatogram of one animal of a species represented the same components as

Table 4. Weight of freeze-dried powder obtained from venison and beef samples. (30 grams for whole muscle tissue and 80 grams for water soluble portion)

Animal number	Location of cut	Yield of dry powder		Actual weight analyzed ^a	
		Whole muscle grams	Water soluble grams	Whole muscle grams	Water soluble grams
<u>Strong venison</u>					
A	Leg ^b	7.3535	3.3900	0.8159	4.7198
A	Leg ^b	6.9552	2.9820	0.8627	5.3655
C	Shoulder	6.5613	2.8184	0.9145	5.6770
		6.9597			
Mean				0.8644	5.2541
<u>Mild venison</u>					
3	Right leg	6.6348	3.1990	0.9043	5.0016
3	Left leg	6.6957	3.2607	0.8961	5.4723
8	Right leg	7.1826	2.9238	0.8354	5.4723
8	Left leg	7.2193	2.8070	0.8311	5.7000
9	Right leg	7.6973	3.9945	0.7795	4.0055
9	Left leg	7.5101	3.6756	0.7989	4.3530
11	Right leg	7.6722	3.8824	0.7820	4.1212
11	Left leg	7.9181	3.7760	0.7578	4.2373
Mean				0.8231	4.7954
<u>Beef</u>					
1	Leg	8.3091	3.5383	0.7221	4.5219
2	Leg	7.7953	3.5881	0.7696	4.4592
3	Leg	8.3166	3.6891	0.7215	4.3371
4	Leg	8.6163	3.4195	0.6964	4.6791
Mean				0.7274	4.4993

^aEquivalent weight of meat represented by 0.2 grams of dried powder used to obtain gas chromatograms.

^bLeg samples from the same animal, but left and right sides were not labeled.

similar peaks in a chromatogram of another animal of the same or different species was empirical. Absolute retention time for each peak was variable and could not be duplicated either between samples or within the same samples. However, the over-all similarity and timing strongly suggests that similar compounds are involved. Considerable work was done to improve the precision of timing in this study but the precision could still be improved.

When peak 2 was used as a reference point for comparison of the time intervals at which the peaks emerged on each chromatogram, the time intervals between peaks were almost identical as shown in Tables 5 through 8.

No attempt was made to identify by chemical analyses the various peaks obtained on the gas chromatograms.

Mild venison versus beef

Gas chromatograms of the volatile compounds obtained from mild venison and beef were found to be essentially identical for the whole muscle analysis or the water soluble portion (Figures 2 to 5). Minor differences were observed occasionally, but they were inconsistent and could not be reproduced. Mean peak areas for the whole muscle tissue and the water soluble portion of mild venison from both the right and left side of the animal, and beef are shown in Table 9. Hornstein and Crowe (1960) working with beef and pork concluded that a similar basic meaty flavor is obtained on heating the lean of beef and pork, while the characteristic

Insert V
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Table 5. Time interval between peaks of chromatograms from mild venison (right leg) when peak 2 was used as a reference point

Animal number		Time interval in minutes between peaks					
		2-3	3-4	4-5	5-6	6-7	2-7
		<u>Water soluble portion</u>					
3	Minutes	-----	2.44	0.71	2.91	6.85	12.91 ^b
	Per cent	-----	18.90	5.50	22.54	53.06	
8	Minutes	1.89	2.52	0.71	3.23	6.61	14.96
	Per cent	12.63	16.85	4.75	21.59	44.19	
9	Minutes	1.73	2.44	0.71	-----	9.53 ^a	14.41
	Per cent	12.01	16.93	4.93	-----	66.14	
11	Minutes	1.81	2.75	0.71	-----	9.37 ^a	14.64
	Per cent	12.36	18.78	4.85	-----	64.00	
Mean	Minutes	1.81	2.54	0.71	-----	9.63 ^a	14.69
	Per cent	12.32	17.29	4.83	-----	65.56	
		<u>Whole muscle tissue</u>					
3	Minutes	1.73	2.75	0.79 ^o	3.47	6.93	15.67
	Per cent	11.04	17.55	5.04	22.14	44.23	
8 ^c	Minutes	2.28	-----	-----	-----	-----	
	Per cent	-----	-----	-----	-----	-----	
9	Minutes	1.81	2.75	0.71	-----	10.32 ^a	15.59
	Per cent	11.61	17.64	4.55	-----	66.20	
11	Minutes	1.97	2.68	0.71	-----	9.76 ^a	15.12
	Per cent	13.03	17.73	4.70	-----	64.55	
Mean	Minutes	1.84	2.73	0.74	-----	10.16 ^a	15.47
	Per cent	11.89	17.65	4.78	-----	65.59	

^aTime interval between peaks 5 and 7.

^bTime interval between peaks 3 and 7.

^cOmitted from calculation.

Table 6. Time interval between peaks of chromatograms from mild venison (left leg) when peak 2 was used as a reference point

Animal number		Time interval in minutes between peaks					
		2-3	3-4	4-5	5-6	6-7	2-7
		<u>Water soluble portion</u>					
3	Minutes	1.89	2.44	0.79	-----	9.76 ^a	14.88
	Per cent	12.70	16.40	5.31	-----	65.59	
8	Minutes	2.21	2.59	0.71	3.23	6.54	15.28
	Per cent	14.46	16.95	4.65	21.14	42.80	
9	Minutes	2.05	2.44	0.79	-----	10.08 ^a	15.36
	Per cent	13.35	15.89	5.14	-----	65.63	
11	Minutes	1.97	2.52	0.71	3.46	6.38	15.04
	Per cent	13.10	16.76	4.72	23.01	42.42	
Mean	Minutes	2.03	2.55	0.75	-----	9.86 ^a	15.14
	Per cent	13.42	16.52	4.93	-----	65.13	
		<u>Whole muscle tissue</u>					
3	Minutes	2.76	2.76	0.71 ^c	3.70	6.38	16.31
	Per cent	16.92	16.92	4.64	22.69	39.12	
8	Minutes	1.97	2.76	0.71	2.60	7.40	15.44
	Per cent	12.76	17.88	4.60	16.84	47.93	
9	Minutes	1.89	2.68	0.79	2.68	7.72	15.76
	Per cent	11.99	17.01	5.01	17.01	48.99	
11 ^b	Minutes	1.58	-----	-----	-----	-----	
	Per cent	-----	-----	-----	-----	-----	
Mean	Minutes	2.21	2.73	0.74	2.99	7.17	15.84
	Per cent	13.95	17.24	4.67	18.88	45.27	

^aTime interval between peak 5 and 7.

^bOmitted from calculation

Table 7. Time interval between peaks of chromatograms from strong venison when peak 2 was used as a reference point

Animal number	Time interval in minutes between peaks						
	2-3	3-4	4-5	5-6	5-7	2-7	
<u>Water soluble portion</u>							
A ₂	Minutes	2.28	2.83	0.71	-----	10.95 ^a	16.77
	Per cent	13.60	16.88	4.23	-----	65.30	
C	Minutes	1.97	2.83	0.71	3.70	6.46	15.67
	Per cent	12.57	18.06	4.53	23.61	41.23	
Mean	Minutes	2.13	2.83	0.71	-----	10.56	16.23
	Per cent	13.09	17.47	4.38	-----	65.07	
<u>Whole muscle tissue</u>							
A ₂	Minutes	1.65	2.52	0.71	-----	9.92 ^a	14.80
	Per cent	11.15	17.03	4.80	-----	67.03	
C	Minutes	1.97	2.68	0.71	-----	10.07 ^a	15.43
	Per cent	12.77	17.39	4.60	-----	65.26	
Mean	Minutes	1.81	2.60	0.71	-----	10.00 ^a	15.12
	Per cent	11.96	17.21	4.70	-----	66.15	

^aTime interval between peaks 5 and 7.

Table 8. Time interval between peaks of chromatograms from beef when peak 2 was used as a reference point

Animal number		Time interval in minutes between peaks					
		2-3	3-4	4-5	5-6	6-7	2-7
		<u>Water soluble portion</u>					
1	Minutes	1.97	2.68	0.79	-----	9.61 ^a	15.05
	Per cent	13.09	17.81	5.25	-----	63.85	
2	Minutes	1.89	2.60	0.71	3.46	6.14	14.80
	Per cent	12.77	17.57	4.80	23.38	41.49	
3	Minutes	1.89	2.68	0.71	3.23	6.22	14.73
	Per cent	12.83	18.19	4.82	21.93	42.23	
4	Minutes	2.05	2.68	0.71	3.70	6.14	15.28
	Per cent	13.42	17.54	4.65	24.22	40.18	
Mean	Minutes	1.95	2.66	0.73	-----	9.63 ^a	14.97
	Per cent	13.03	17.78	4.88	-----	64.33	
		<u>Whole muscle tissue</u>					
1	Minutes	1.89	2.83	0.71	-----	10.00 ^a	15.43
	Per cent	12.25	18.34	4.60	-----	64.81	
2	Minutes	1.89	2.83	0.63	3.46	5.97	14.72
	Per cent	12.84	19.23	4.28	23.51	40.15	
3	Minutes	2.20	2.28	0.71	-----	10.32 ^a	15.51
	Per cent	14.18	14.70	4.58	-----	66.54	
4	Minutes	1.73	2.91	0.79	2.76	7.48	15.67
	Per cent	11.04	18.57	5.04	17.61	47.74	
Mean	Minutes	1.93	2.71	0.72	-----	9.98 ^a	15.34
	Per cent	12.58	17.67	4.69	-----	65.06	

^aTime interval between peaks 5 and 7.

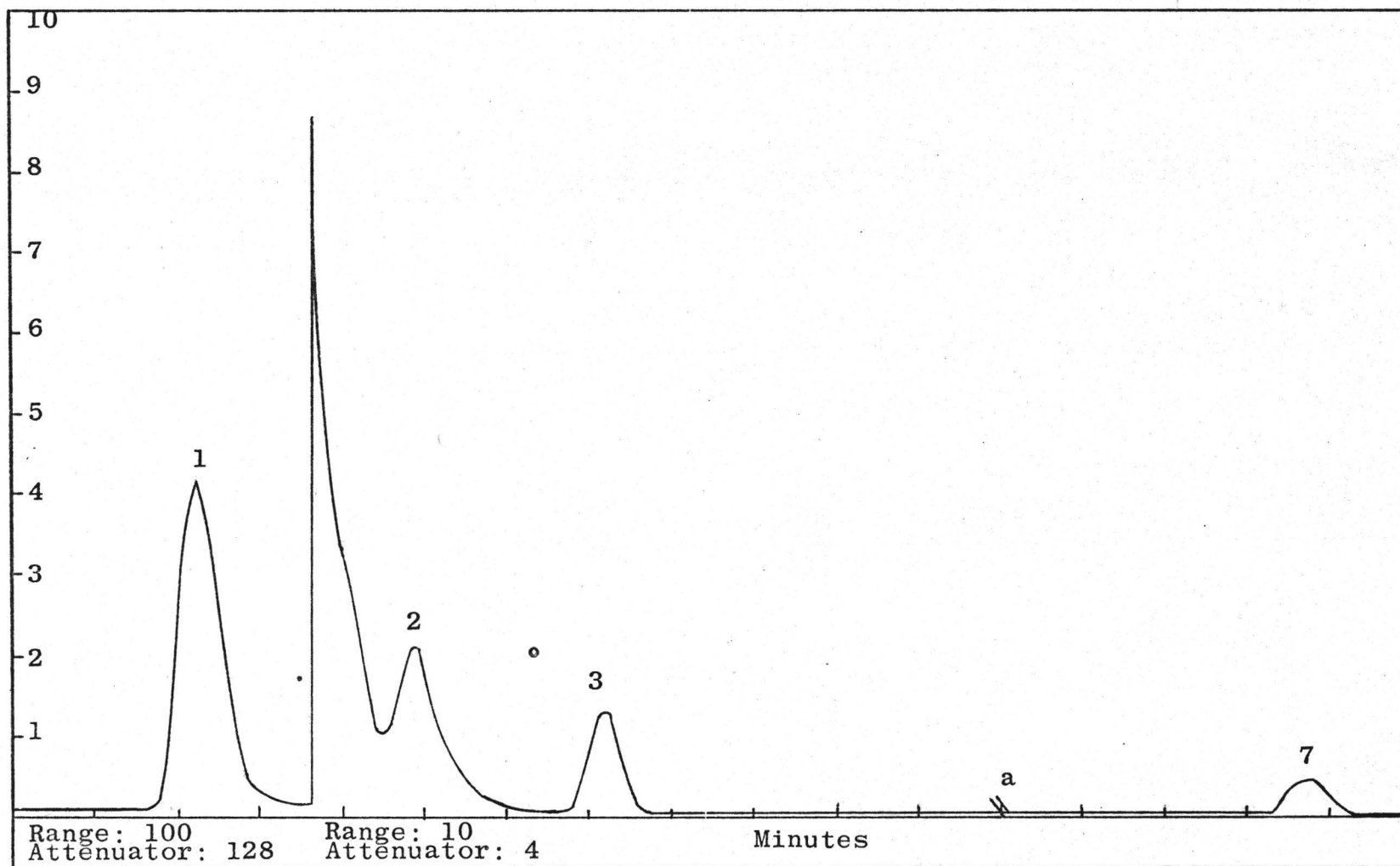


Figure 2. Gas chromatograms of volatile compounds isolated from whole muscle tissue of mild venison sample No. 8, right leg

^aFold equivalent to 6 minutes run.

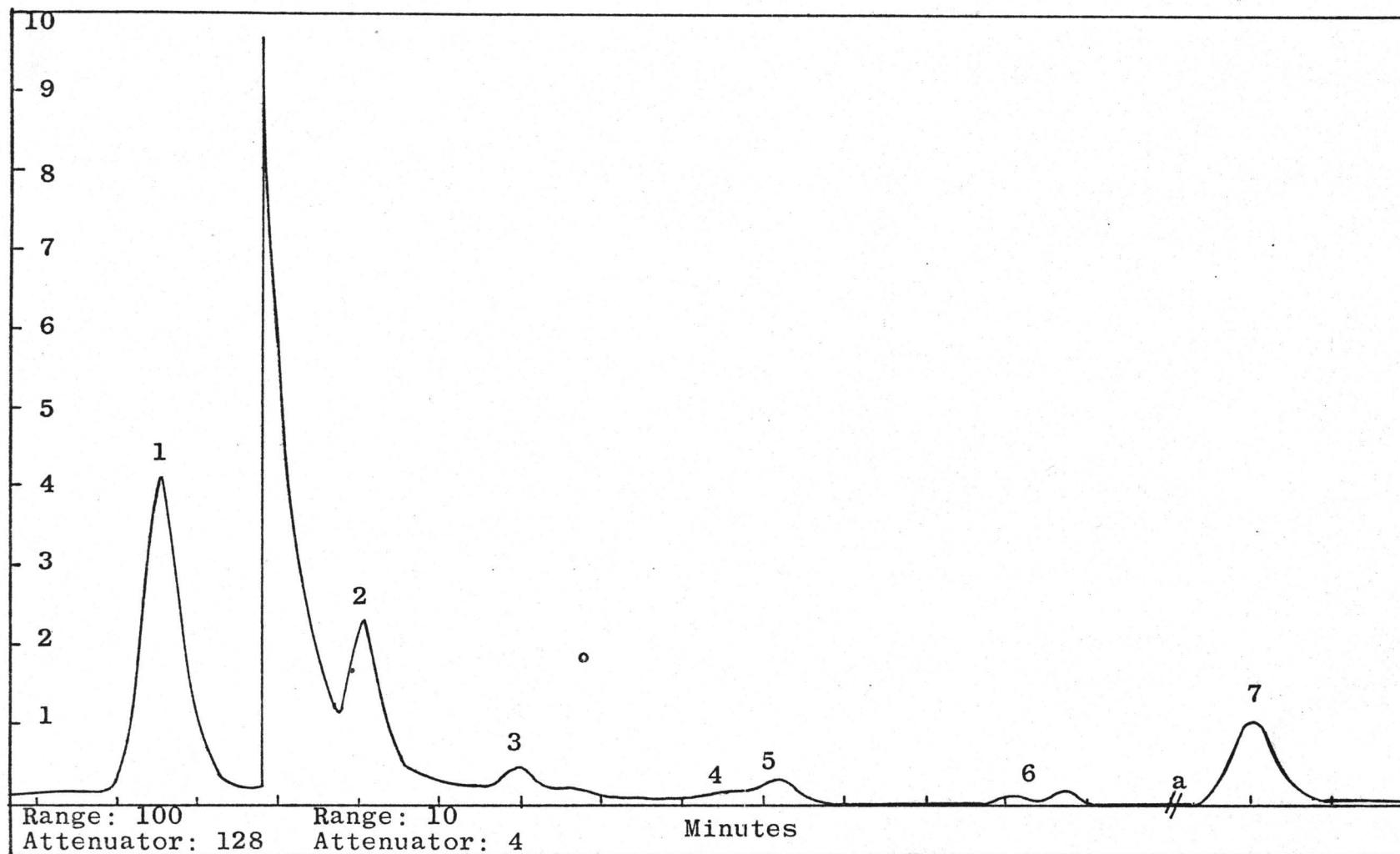


Figure 3. Gas chromatogram of volatile compounds isolated from water soluble portion of muscle of mild venison sample No. 8, right leg

^aFold equivalent to 4 minutes run.

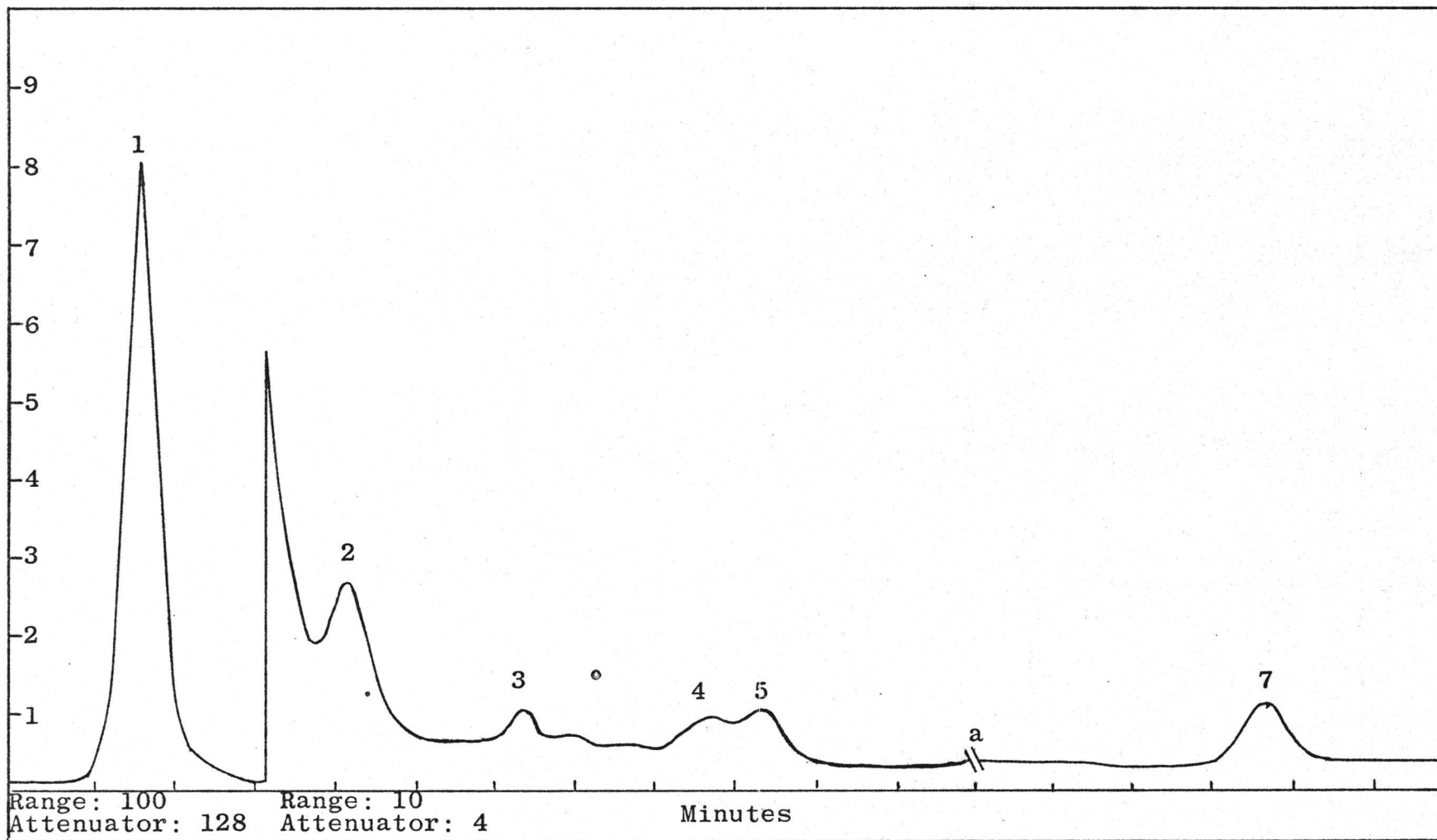


Figure 4. Gas chromatogram of volatile compounds isolated from whole muscle tissue of beef sample No. 3, leg

^aFold equivalent to 4 minutes run.

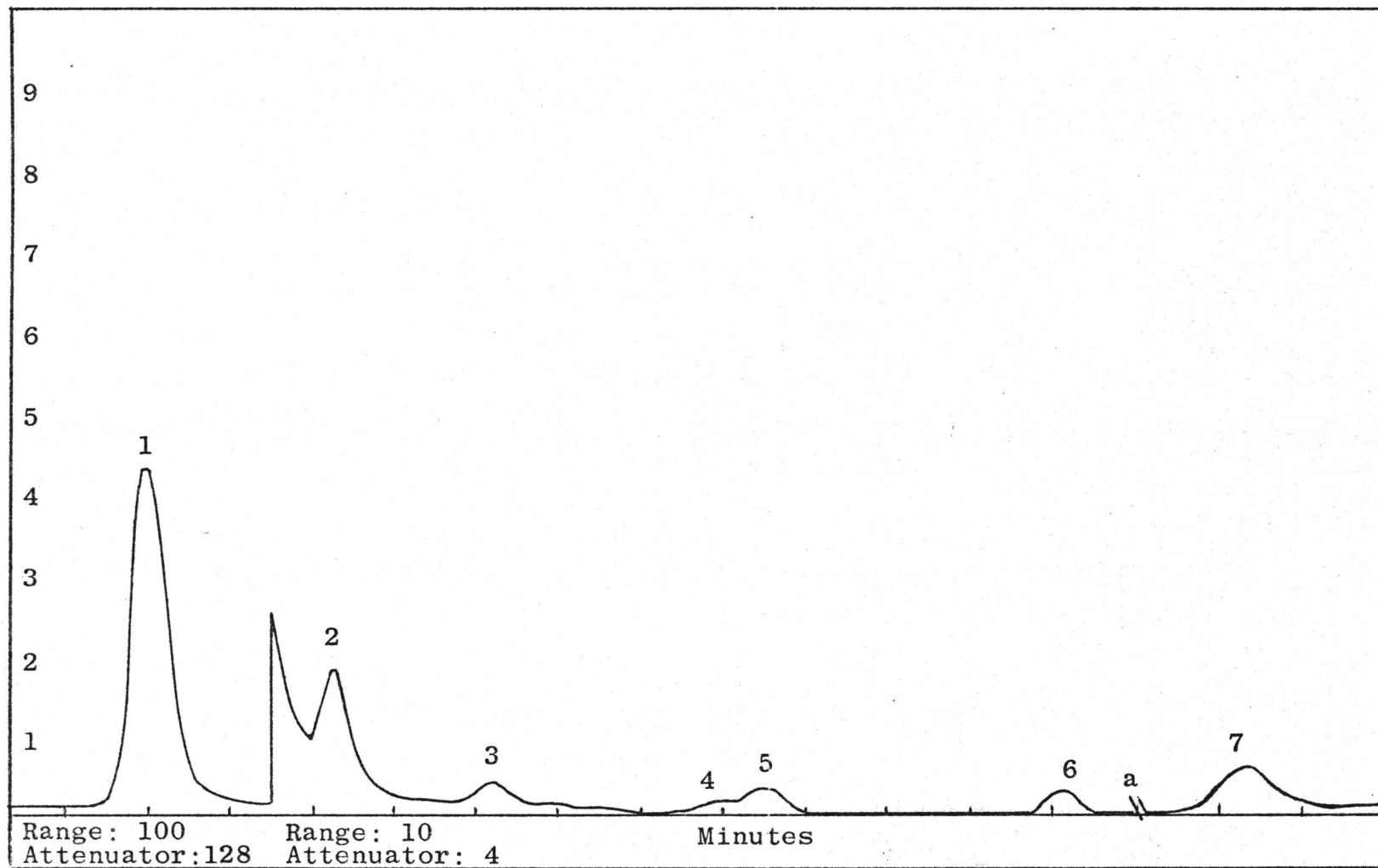


Figure 5. Gas chromatogram of volatile compounds isolated from water soluble portion of muscle of beef sample No. 3, leg

^aFold equivalent to 4 minutes run.

Table 9. Mean peak areas for volatile components from chromatograms of beef and venison samples

Animal	Mean peak values in square centimeters							Actual weight analyzed in grams
	1	2	3	4	5	6	7	
<u>Water soluble portion</u>								
Beef	4.27	0.89	0.33	0.12	0.13	0.34	0.98	4.4993
Mild venison (left)	3.55	0.90	0.63	0.21	0.17	0.08	0.58	4.7954
Mild venison (right)	3.85	0.82	1.19	0.05	0.15	0.30	0.90	4.7954
Strong venison	6.71	1.58	0.30	0.05	0.24	0.02	0.48	5.2541
<u>Whole muscle tissue</u>								
Beef	5.50	1.46	0.84	0.15	0.31	0.33	0.83	0.7274
Mild venison (left)	4.92	1.07	0.09	0.05	0.09	0.05	0.72	0.8231
Mild venison (right)	4.75	0.97	1.30	0.05	0.08	0.01	1.19	0.8231
Strong venison	5.09	1.54	0.18	0.12	0.54	----	0.89	0.8644

<u>Ether Soluble Portion</u>								
	A	1	B	2	3	4	5	7
mild venison	0.10	2.89	2.52	1.64	1.96	0.07	0.10	0.26
strong venison	0.55	4.60	3.02	1.20	1.28	0.06	0.16	0.28

flavor differences in pork and beef reside in the fat.

Strong venison versus mild venison

The individual deer were classified by a panel of judges as having mild or strong venison flavor from the ratings on leg roasts. From a total of 12 deer, the 6 animals used in this study were selected. Meat from animals 1 to 4 were rated mild while that from A and C were judged to have a somewhat strong venison flavor. However, neither A or C were rated as having a real strong venison flavor. An attempt was made to obtain such a deer but due to the season, age of animals, or some unknown factor, a deer with a real strong venison flavor could not be obtained during the 1962 season.

When the values for the strong venison were compared to those of the mild venison, differences were shown only in the peak areas from the water soluble portions. Mean values are shown in Table 9 and individual values for the several deer are shown in Tables 10 and 11; Figures 3 and 6.

Strong venison had ^{somewhat} larger peak areas for peaks 1, 2, and 5 than those of mild venison while the reverse was true for the other peaks. This is shown clearly in Figures 3 and 6. Whether these differences in peak areas represented differences in kind and amounts of volatile components inherent in the strong venison as compared to the mild venison could not be determined in this study. Identification of kind and amount of each compound must await further investigation.

Table 10. Peak areas for volatile components from chromatograms of the water soluble portion of muscle of mild venison

Animal number	Peak values in square centimeters							Actual weight analyzed in grams
	1	2	3	4	5	6	7	
<u>Right leg</u>								
3	4.56	0.34	0.23	0.08	0.27	1.13	1.36	5.0016
8	4.22	0.96	0.14	0.03	0.15	0.08	0.82	5.4723
9	2.79	1.09	3.60	0.05	----	----	0.18	4.0055
11	3.83	0.90	0.79	0.03	0.18	----	1.23	4.1212
Mean	3.85	0.82	1.19	0.05	0.15	0.30	0.90	
<u>Left leg</u>								
3	3.54	0.35	0.13	0.04	0.03	0.02	0.25	5.4723
8	5.15	2.07	0.71	0.34	0.28	0.11	1.07	5.7000
9	2.36	0.50	0.61	0.38	0.25	----	0.56	4.3530
11	3.15	0.67	1.05	0.07	0.11	0.17	0.43	4.2373
Mean	3.55	0.90	0.63	0.21	0.17	0.08	0.58	

Table 11. Peak areas for volatile components from chromatograms of muscle of strong venison

Animal number	Peak values in square centimeters							Actual weight analyzed in grams
	1	2	3	4	5	6	7	
	<u>Water soluble portion</u>							
A ^a	8.4	2.27	0.39	0.04	0.25	----	0.42	4.7198
C ^b	5.01	0.90	0.22	0.05	0.22	0.03	0.54	5.3655
Mean	6.71	1.58	0.30	0.05	0.24	0.02	0.48	
	<u>Whole muscle tissue</u>							
A ^a	6.17	1.83	0.56	0.19	0.81	----	1.75	0.8159
C ^b	4.00	1.25	0.16	0.05	0.29	----	1.82	0.9145
Mean	5.09	1.54	0.18	0.12	0.54	----	0.89	

^aLeg muscle

^bShoulder muscle

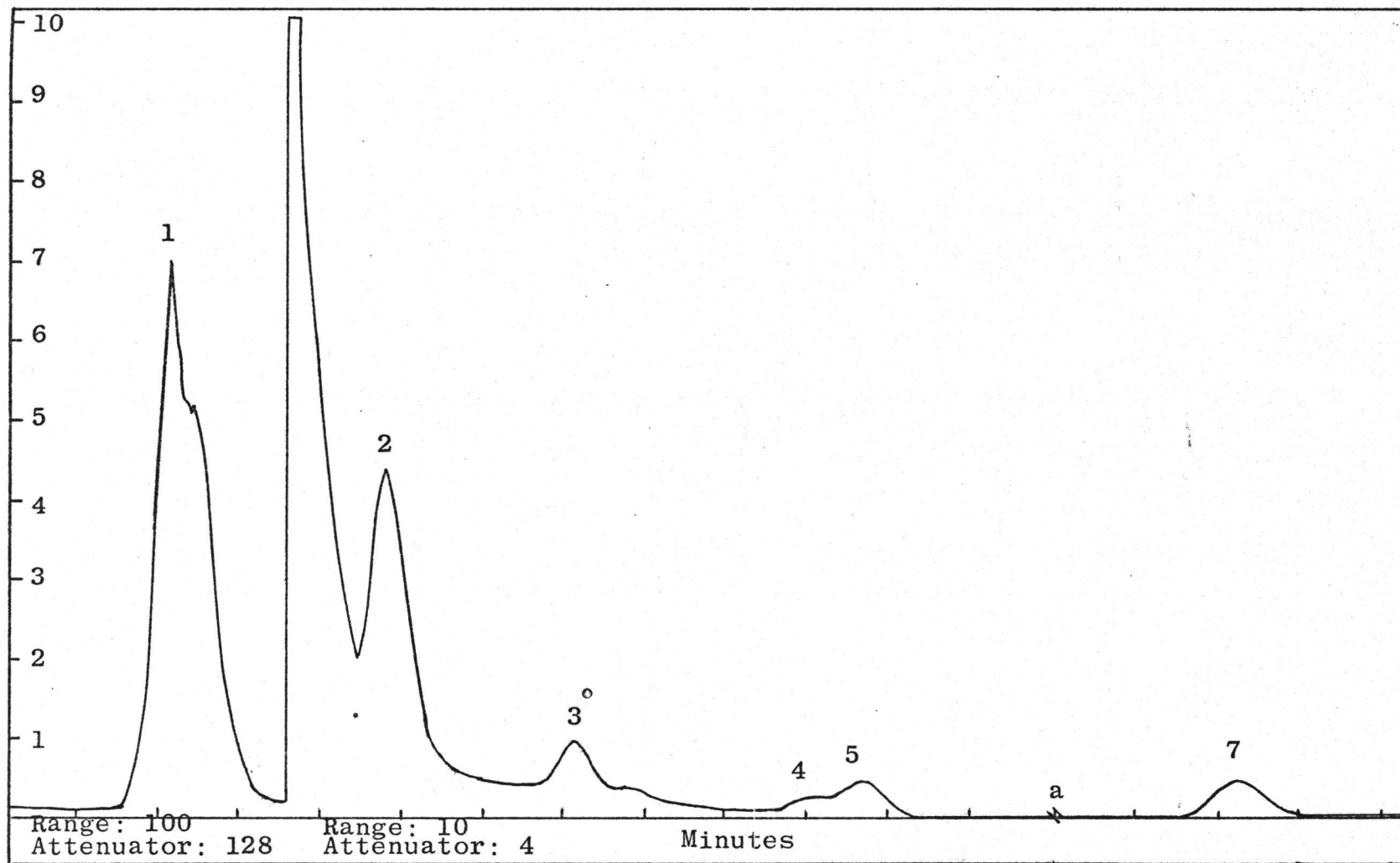


Figure 6. Gas chromatogram of volatile compounds isolated from water soluble portion of muscle of strong venison sample No. A, leg

^aFold equivalent to 6 minutes run.

Hornstein et al. (1960) found small amounts of carbonyls, ammonia, and hydrogen sulfide in the most volatile fraction.

Similar differences were not observed in peak areas from whole muscle tissue. See Tables 9, 11, and 12 and Figures 2 and 7.

Right leg versus left leg

A comparison of flavor components obtained from the left and right side of the same animal could be made on only the mild venison. The peak area values are shown in Tables 10 and 12 and the gas chromatograms in Figures 2, 3, 8, and 9. Although some differences were observed in the values for the individual animal, mean values were similar for either the whole muscle or the water soluble portion.

Strong venison versus beef

Gas chromatograms of the volatile components obtained from strong venison and beef were found to be similar in pattern and peak areas for the whole muscle tissue (Figures 7, 13, and 4 and Tables 11 and 13. When the peak areas for the water soluble portions were compared (Figures 5, 6, and 11 and Tables 11 and 13) strong venison represented a higher concentration in peaks 1 and 2 than was found for beef.

Here again, the possible differences in strong venison and beef could be due to the concentration or kind of components appearing at peaks 1 and 2.

Table 12. Peak areas for volatile components from chromatograms of the whole muscle tissues of mild venison

Animal number	Peak areas in square centimeters							Actual weight analyzed in grams
	1	2	3	4	5	6	7	
<u>Right leg</u>								
3	5.68	0.86	0.08	0.14	0.17	0.05	2.42	0.9043
8	4.53	1.09	0.12	----	----	----	0.38	0.8354
9	3.60	0.76	4.56	0.04	0.08	----	0.60	0.7795
11	5.19	1.16	0.45	0.02	0.07	----	1.35	0.7820
Mean	4.75	0.97	1.30	0.05	0.08	0.01	1.19	
<u>Left leg</u>								
3	6.42	1.12	0.06	0.04	0.09	0.04	1.08	0.8961
8	5.42	1.63	0.08	0.02	0.07	0.03	0.66	0.8311
9	4.70	0.93	0.20	0.14	0.18	0.11	0.96	0.7989
11	3.12	0.60	0.01	----	----	----	0.16	0.7578
Mean	4.92	1.07	0.09	0.05	0.09	0.05	0.72	

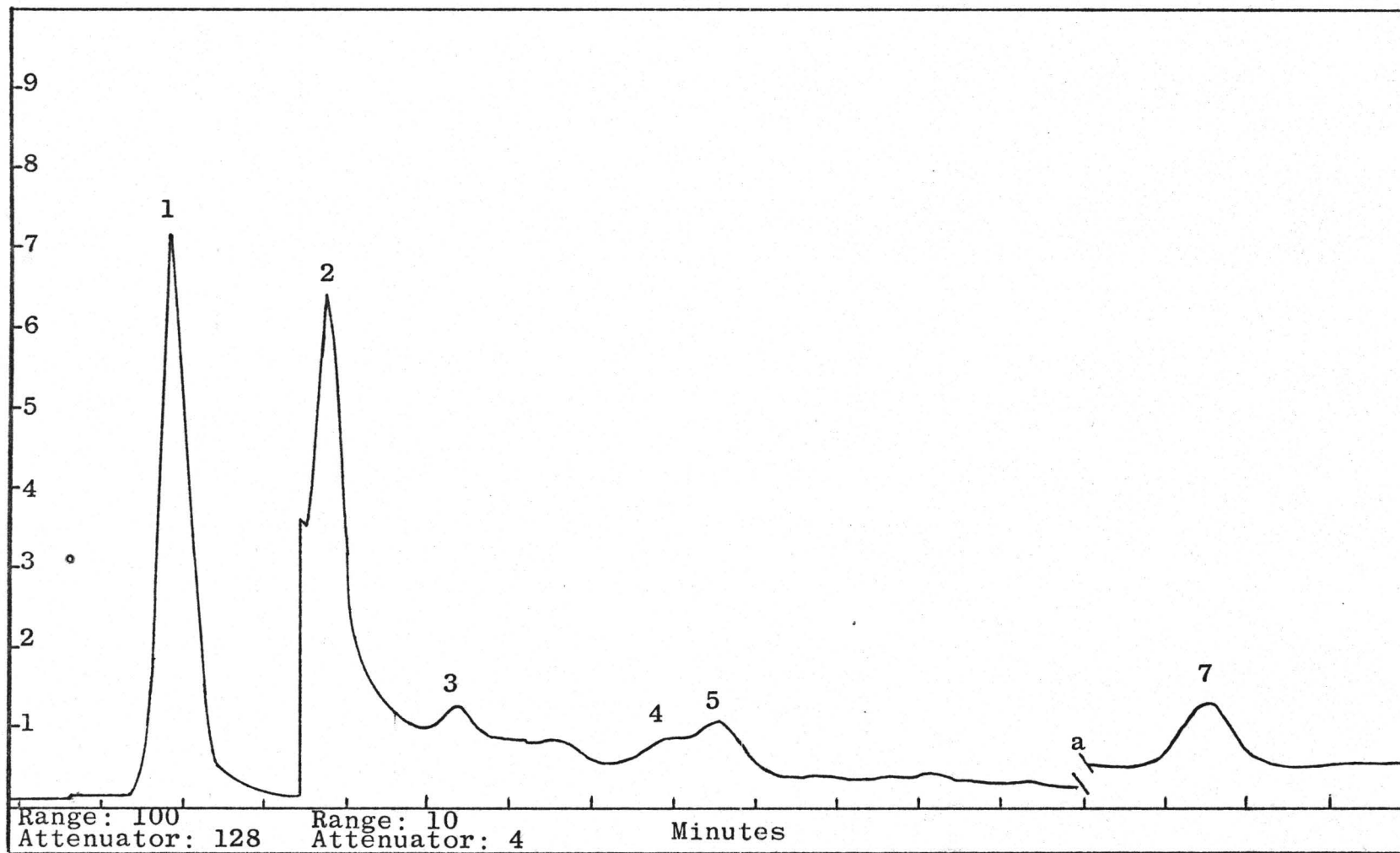


Figure 7. Gas chromatogram of volatile compounds isolated from whole muscle tissue of strong venison sample No. A, leg

^aFold equivalent to 4 minutes run.

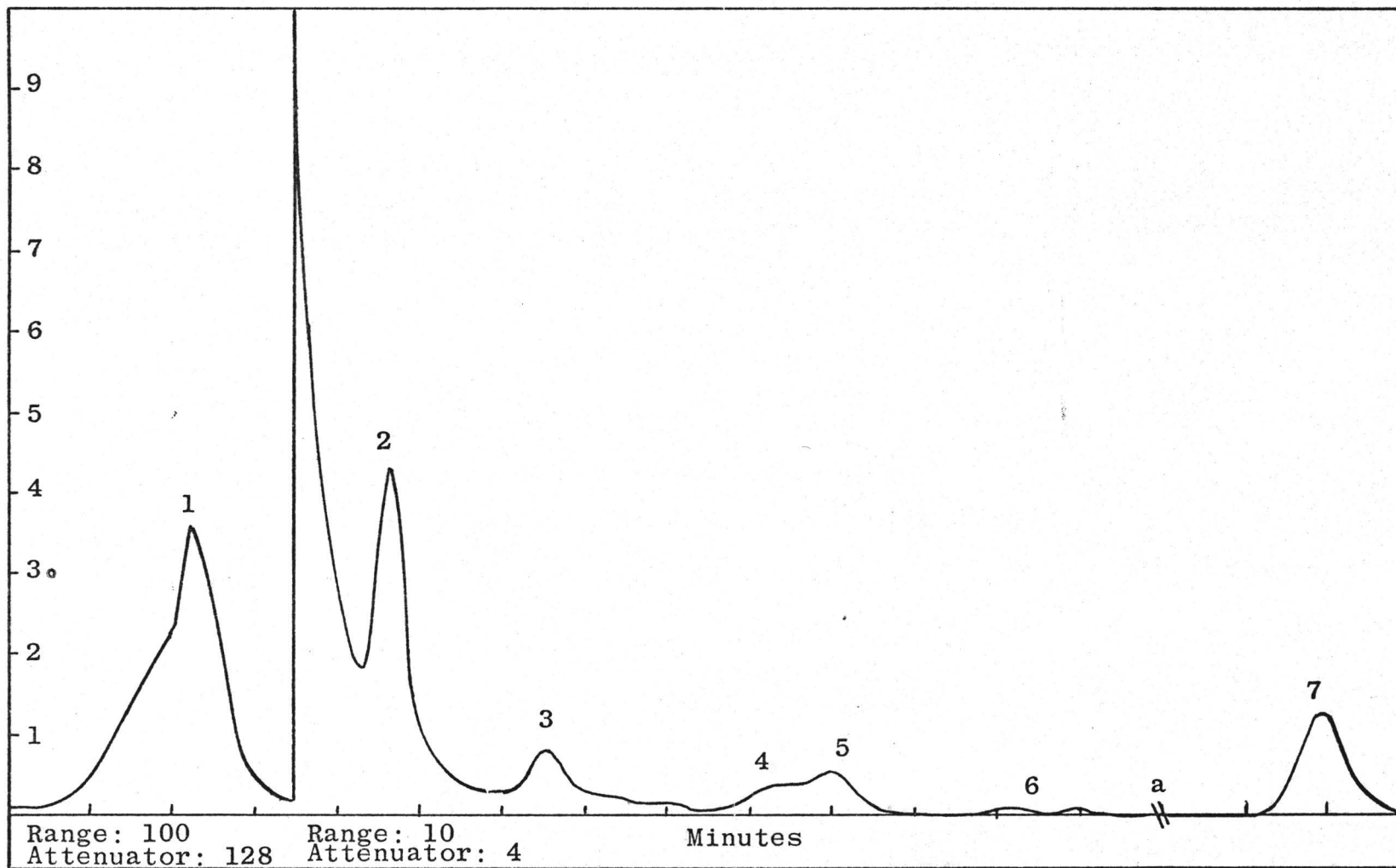


Figure 8. Gas chromatogram of volatile compounds isolated from water soluble portion of muscle of mild venison sample No. 8, left leg

^aFold equivalent to 4 minutes run.

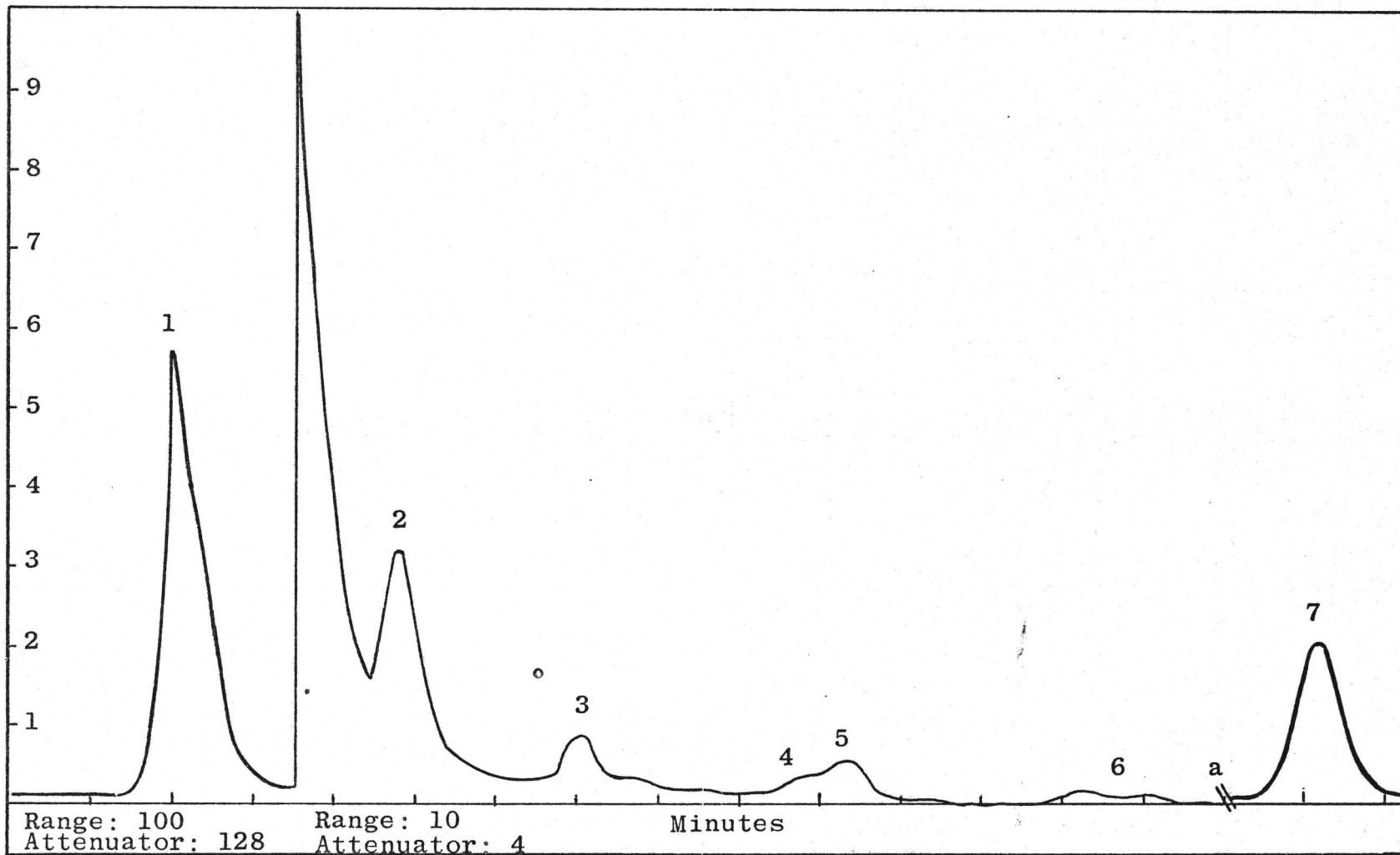


Figure 9. Gas chromatogram of volatile compounds isolated from whole muscle tissue of mild venison sample No. 8, left leg

^aFold equivalent to 4 minutes run.

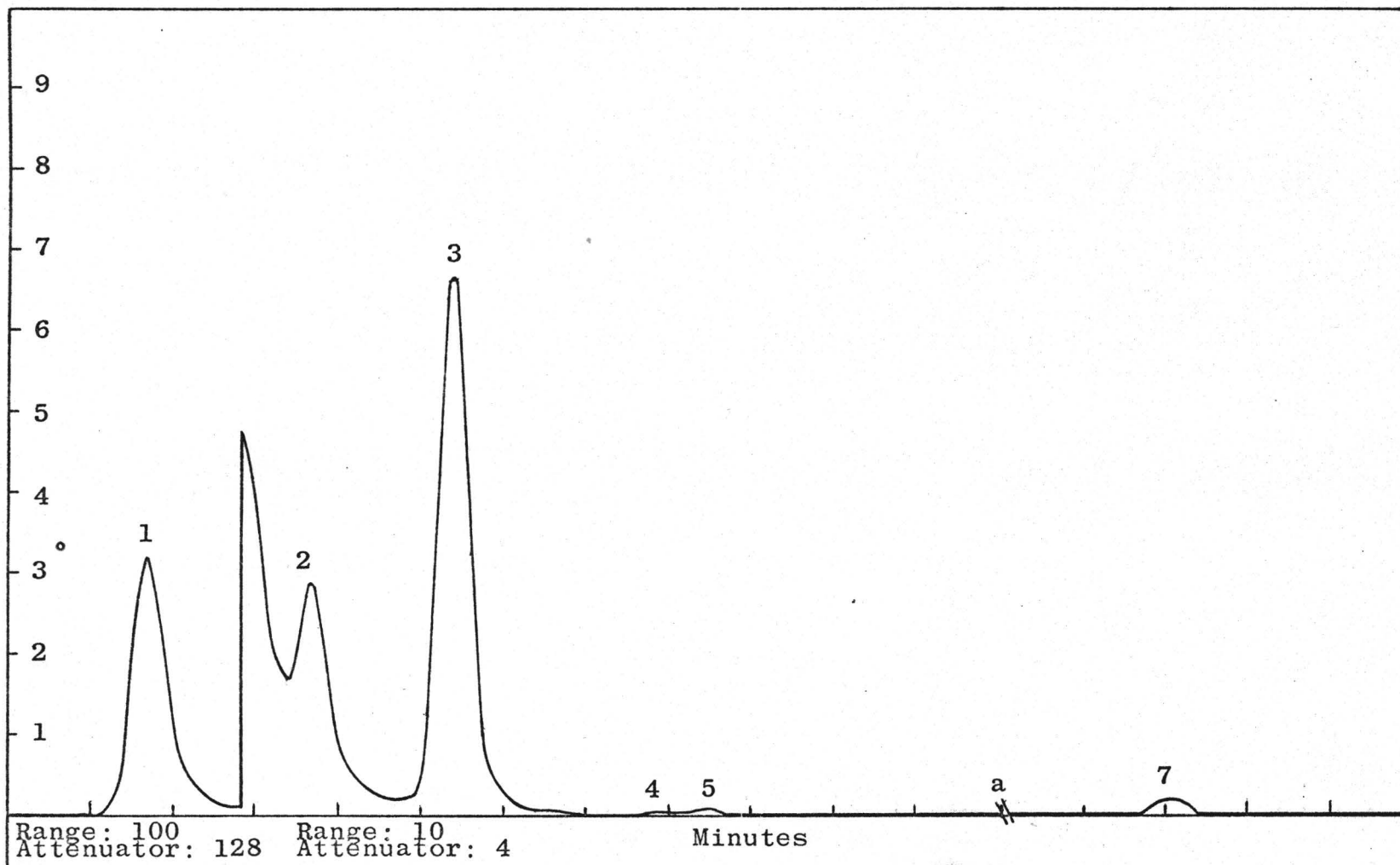


Figure 10. Gas chromatogram of volatile compounds isolated from water soluble portion of muscle of mild venison sample No. 9, right leg

^aFold equivalent to 4 minutes run.

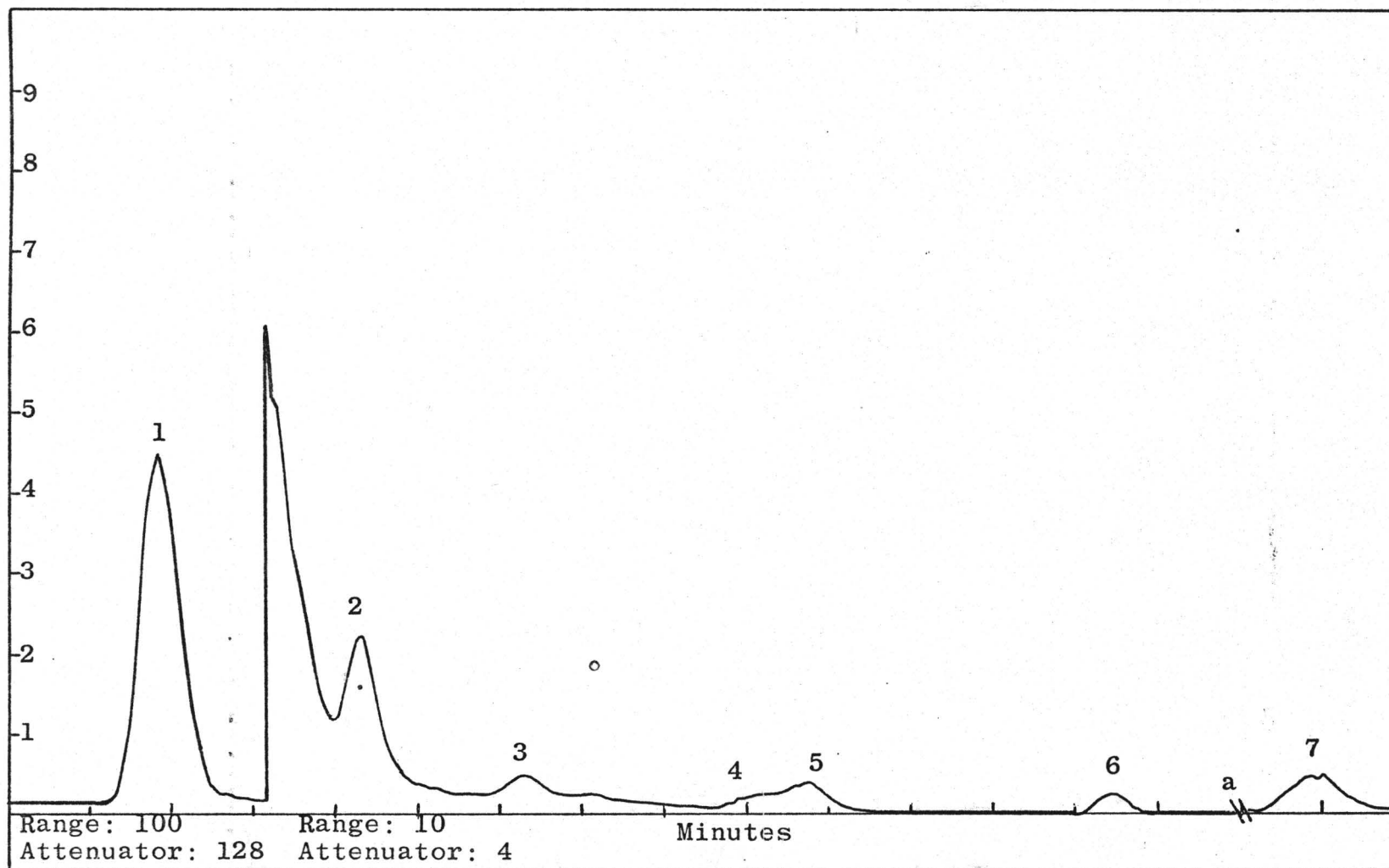


Figure 11. Gas chromatogram of volatile compounds isolated from water soluble portion of muscle of strong venison sample No. C, shoulder

^a Fold equivalent to 4 minutes run.

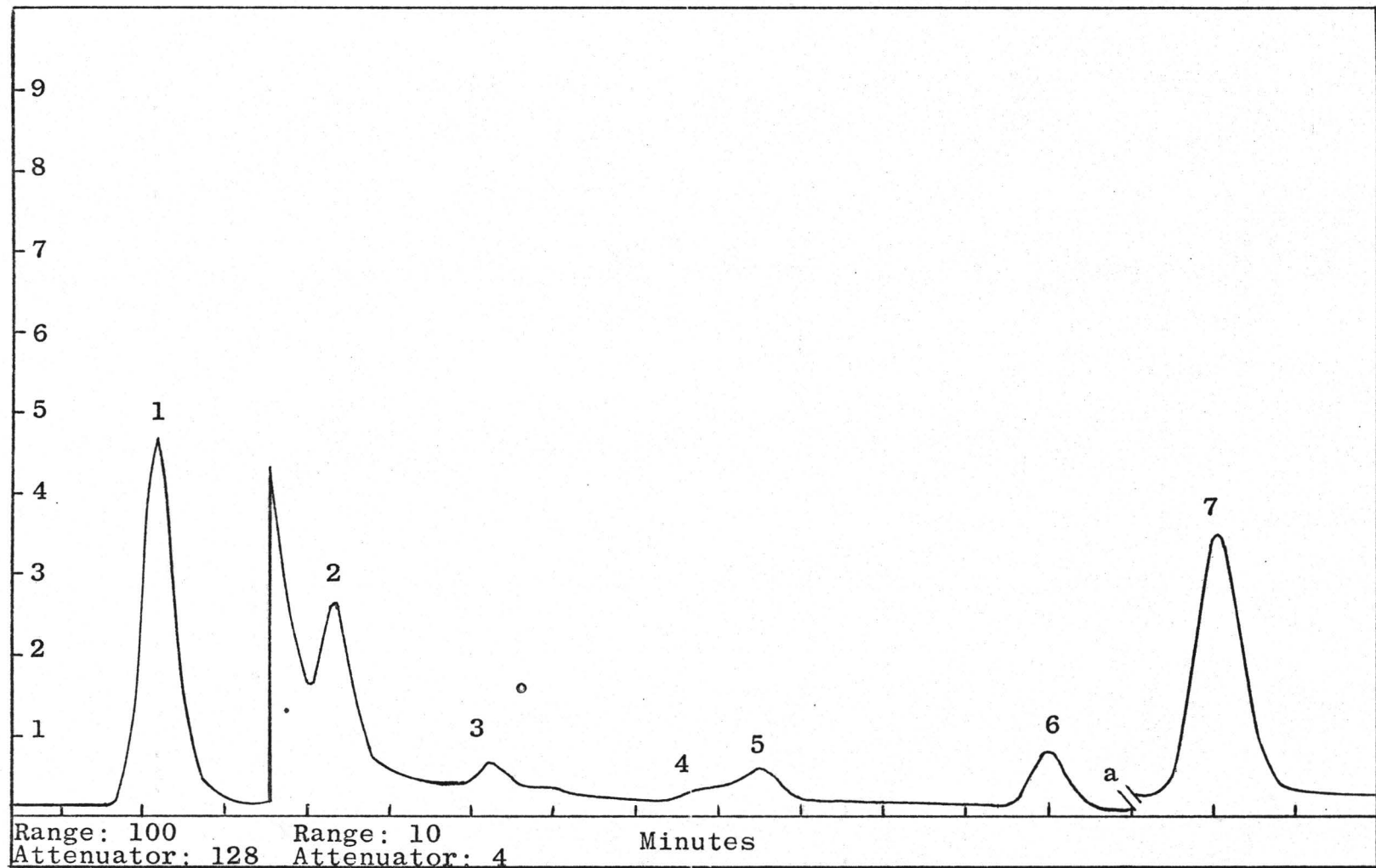


Figure 12. Gas chromatogram of volatile components isolated from water soluble portion of muscle of beef sample No. 2, leg

^aFold equivalent to 4 minutes run.

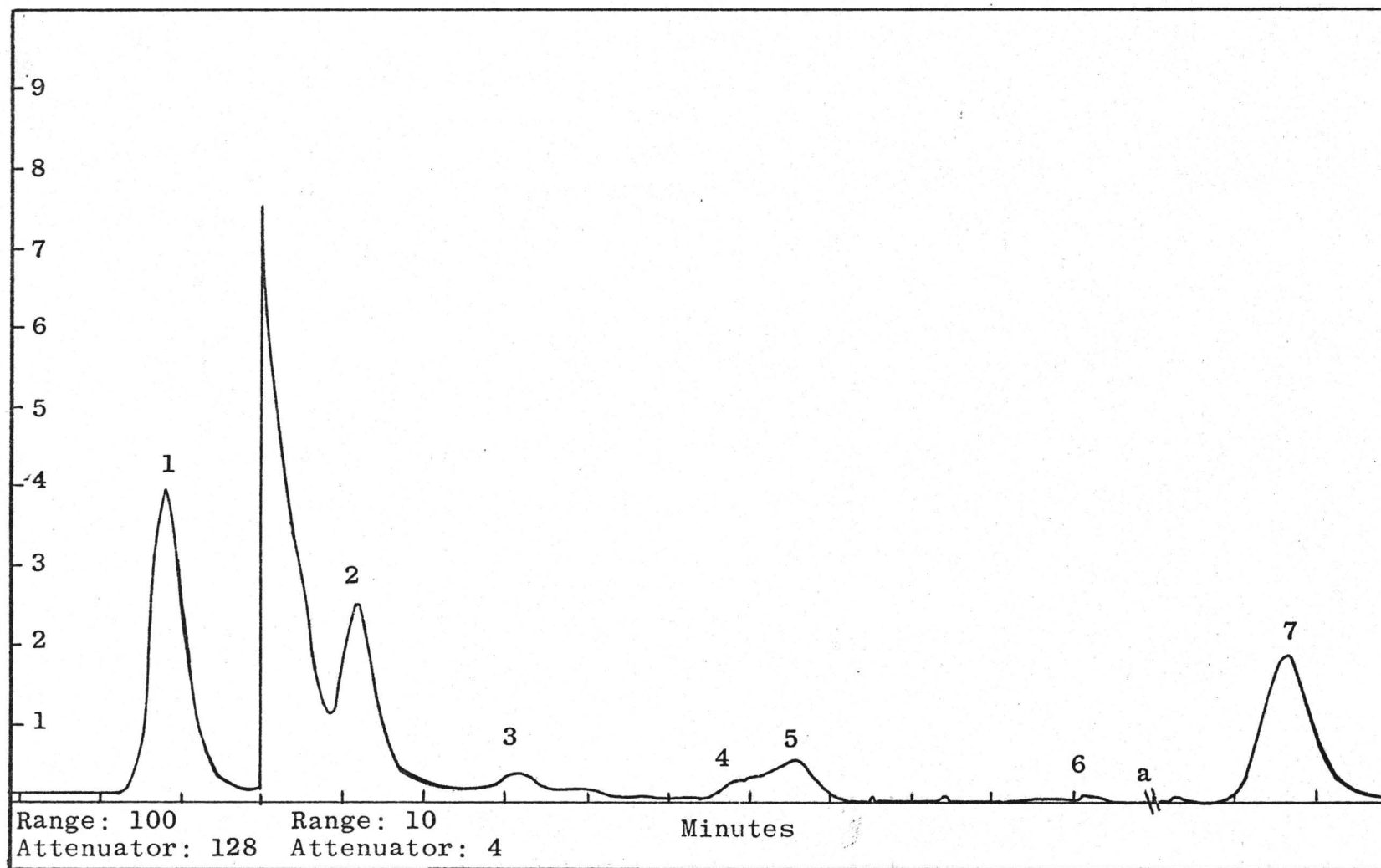


Figure 13. Gas chromatogram of volatile compounds isolated from whole muscle tissue of strong venison sample No. C, shoulder

^aFold equivalent to 4 minutes run.

Variation between animals

Comparisons of volatile components obtained from animals within groups were made. Since the results from water soluble portions were more persistent than the whole muscle tissue, graphs were made for examples of these differences.

When peak areas from animals classified as mild venison were compared, quite wide differences between two animals were observed (Tables 10; Figures 3 and 10). However, the general pattern of the chromatograms were similar.

When the samples of strong venison were compared, Table 11 and Figures 6 and 11, a marked difference was noticed in peak 1 and 2. Thus, the first two peaks were different in leg and shoulder muscles, whereas the other five peaks were more or less identical.

In the beef samples Table 13 and Figures 12 and 5, there was very little difference in peak areas from animal to animal.

Shoulder versus leg

A comparison between values from shoulder and leg of the strong venison, Table 11 and Figures 7 and 13, showed that there was some variation. The areas under the peaks 1 to 6 were different with more from the leg sample than the shoulder. This might indicate that the intensity of the flavor in the leg muscle was greater than in the shoulder muscle.

Table 13. Peak areas for volatile components from chromatograms of the leg samples of beef

Animal number	Peak values in square centimeters							Actual weight analyzed in grams
	1	2	3	4	5	6	7	
<u>Water soluble portion</u>								
1	3.78	1.05	0.54	0.07	0.06	----	0.51	4.5219
2	4.06	0.90	0.29	0.17	0.16	0.72	2.46	4.4592
3	4.27	0.64	0.16	0.05	0.09	0.22	0.50	4.3371
4	4.95	0.98	0.32	0.17	0.19	0.43	0.44	4.4993
Mean	4.27	0.89	0.33	0.12	0.13	0.34	0.98	
<u>Whole muscle tissue</u>								
1	4.43	1.08	1.26	0.05	0.15	0.05	1.59	0.7221
2	4.68	0.88	0.48	0.05	0.25	0.22	3.71	0.7696
3	8.38	1.04	0.80	0.29	0.57	0.71	0.57	0.7215
4	4.54	2.82	0.81	0.19	0.26	0.34	0.44	0.6964
Mean	5.50	1.46	0.84	0.15	0.31	0.33	1.83	

Whole muscle versus water
soluble portions

Before differences between values for the whole muscle and the water soluble portion can be compared, ^{begin} the fact that values for the water soluble portion represents approximately 6 times more meat than the values for the whole meat must be considered, ^{when making comparisons.} Hence, the results indicated that the components in the whole muscle tissue were markedly greater than those in the water soluble portions, ^{to here} (Tables 10 to 13) for mild venison, strong venison, and beef. Insert VII ✓

Comparison of ether soluble portions

^{copy} The ether soluble portion of lyophilized whole muscle ^{Insert VIII} of mild and strong venison showed similar chromatograms and most of the peak areas were similar [Table 14 and Figures 14, 15, and 16). ^{omit} However, two additional peaks, peak A before and peak B following the regular peak 1 appeared. No peak appeared at the time of emergence of regular peak 6. The first three peaks, A, 1, and B, from the leg of the strong venison sample were different from those of the ^{to here} shoulder. }

Comparing chromatograms of mild and strong venisons (Table 14 and Figures 14 and 15), peak B showed a marked difference, that is, ^{begin} there was almost twice as much of the components measured by peak B in the strong venison as found in the mild venison. In peak 1 there was also a somewhat higher concentration from the strong venison ^{to here} as compared to the mild venison. Otherwise all the other peaks were more Insert IX
Copy IX

Table 14. Peak areas for volatile components from chromatograms of the ether soluble portion of venison samples

Animal number	Peak areas in square centimeters								
	A	1	B	2	3	4	5	7	
<u>Strong venison</u>									
A	0.36	3.51	3.54	1.28	1.52	0.08	0.16	0.28	
C	0.73	5.70	2.50	1.12	1.03	0.05	0.18	----	
Mean	0.55 12.8	4.60 106.7	3.02 10.1	1.20 27.8	1.28 29.7	0.07 1.6	0.17 3.9	0.28 6.5	4.3105
<u>Mild venison</u>									
9 (rt.)	0.10	2.89	2.52	1.64	1.96	0.07	0.10	0.26	
3 (lt.)	0.20	4.68	1.08	0.66	0.62	0.01	0.07	0.86	
Mean	0.15 3.7	3.78 92.2	1.80 43.9	1.15 28.0	1.29 31.5	0.04 1.0	0.09 2.2	0.56 13.7	4.1004

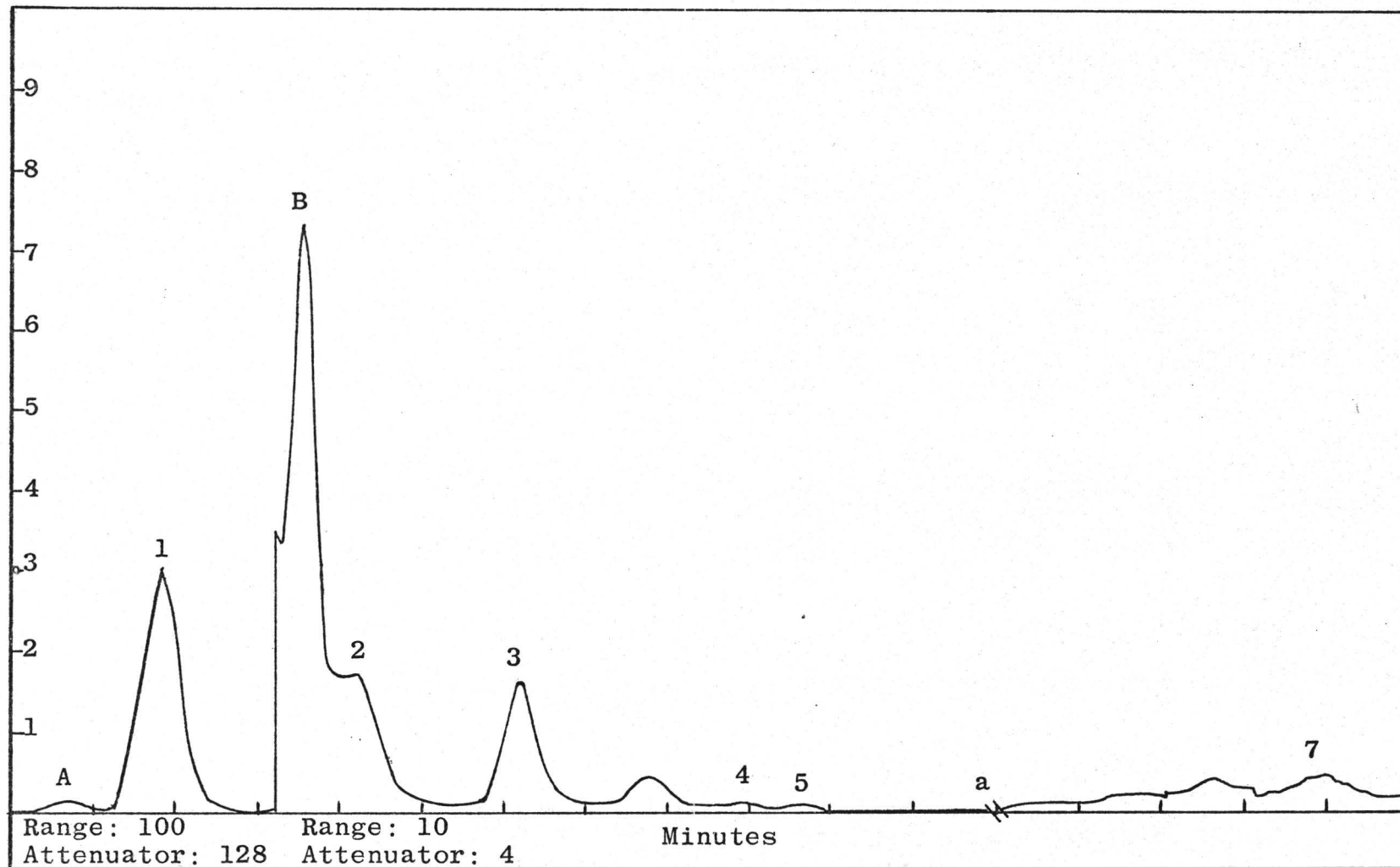


Figure 14. Gas chromatogram of volatile compounds isolated from ether soluble portion of the whole muscle tissue of mild venison sample No. 9, right leg

^aFold equivalent to 4 minutes run.

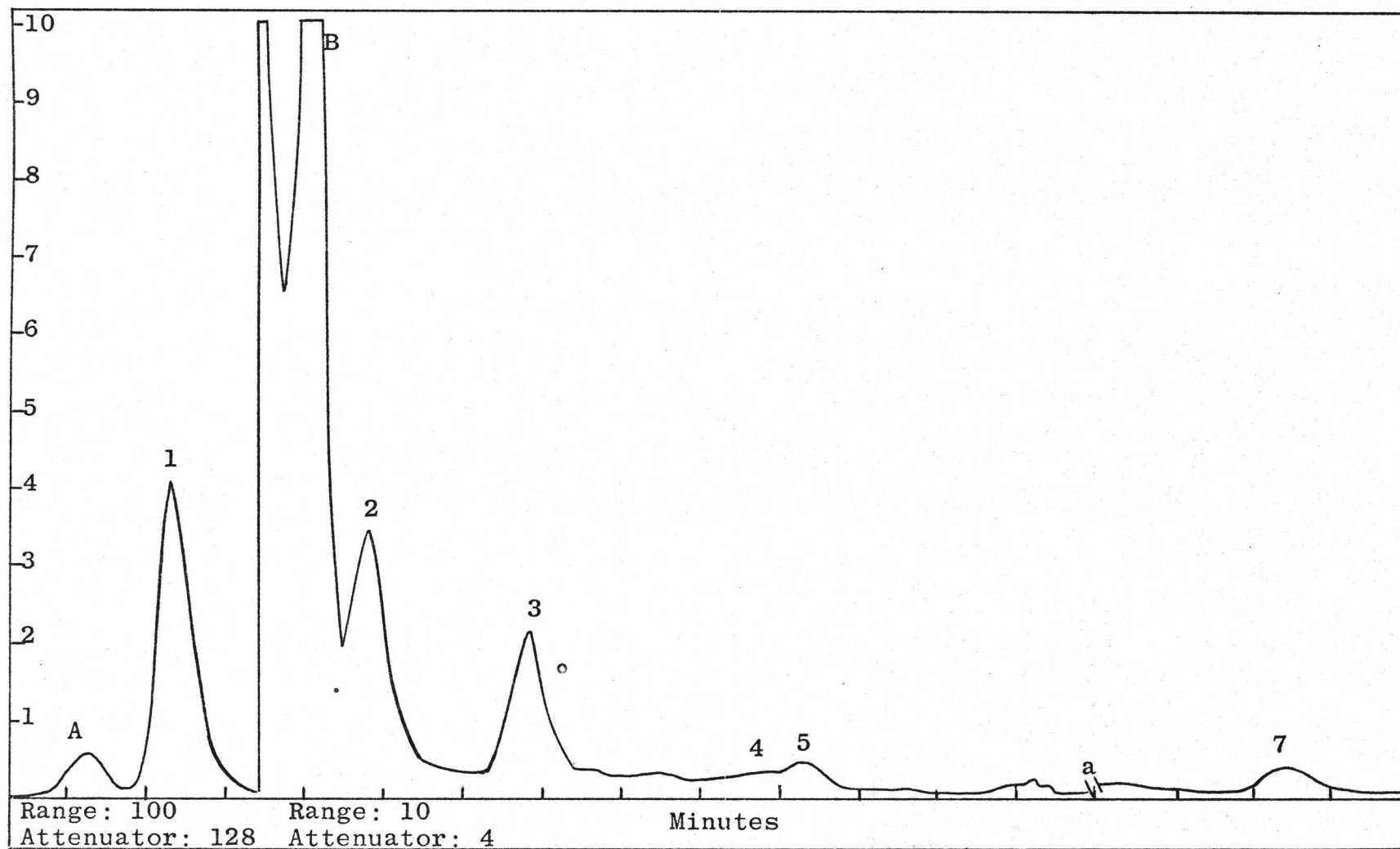


Figure 15. Gas chromatogram of volatile compounds isolated from ether soluble portion of the whole muscle tissue of strong venison sample No. A, leg

^aFold equivalent to 4 minutes run.

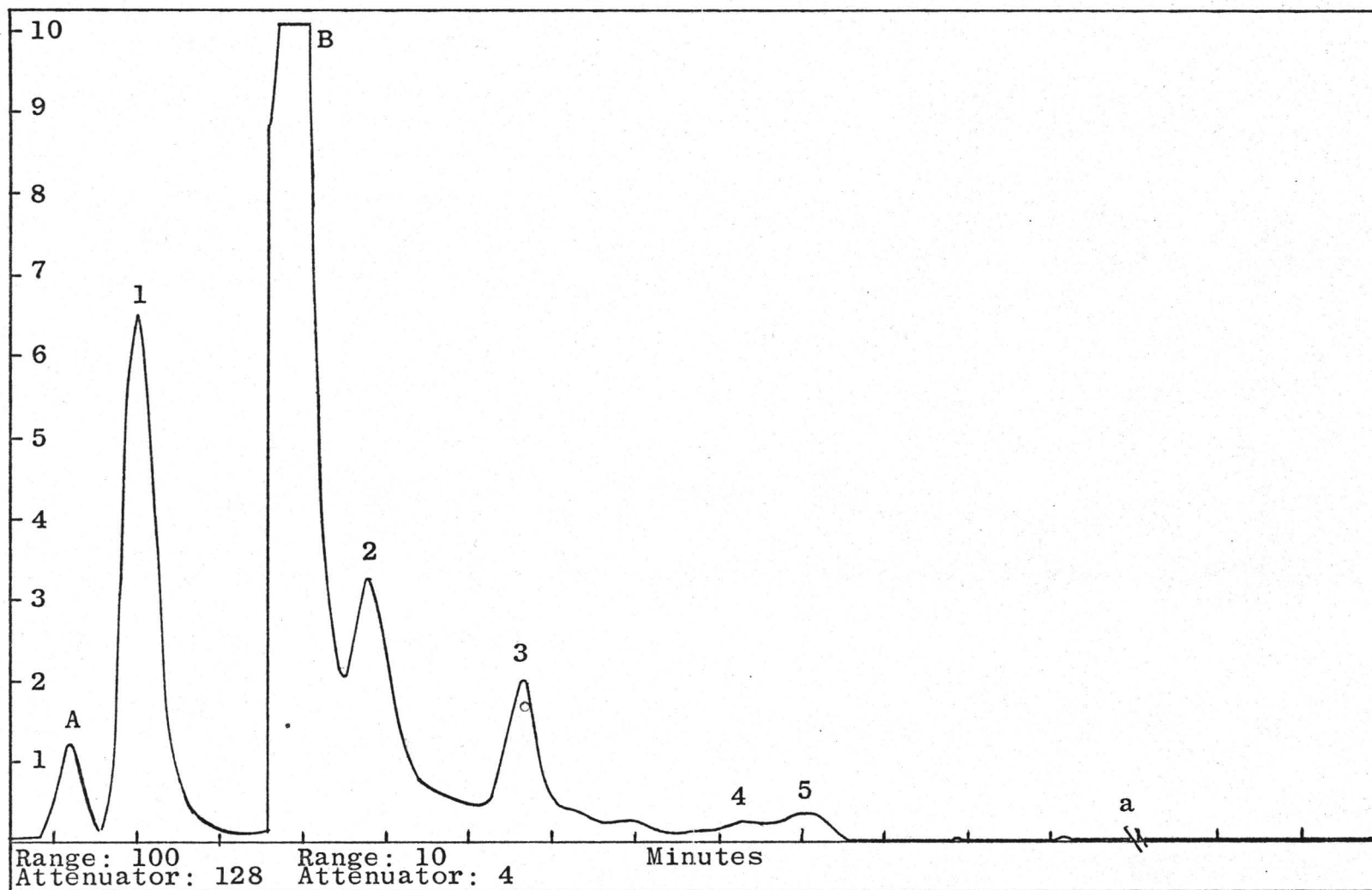


Figure 16. Gas chromatogram of volatile compounds isolated from ether soluble portion of the whole muscle tissue of strong venison sample No. C, shoulder

^aFold equivalent to 4 minutes run.

or less similar.

Glands

In general the peaks obtained from the metatarsal and the tarsal glands occurred at about the same place and were of a similar area except for peak 1 ^{which was much greater than} as those obtained from muscle tissue samples. ^{To here} The first peak area (Table 15 and Figures 17 and 18), in both these chromatograms, was much greater than most other muscle tissue chromatograms. They appeared to be more similar to strong venison samples, than to mild venison. Insert
VII

Sensory tests showed the metatarsal gland to have a more intense odor than the tarsal gland. Comparison of the peaks showed that peak 1 indicated less concentration of volatile components and peaks 2 and 7 more concentration than that shown by the tarsal gland. This finding indicates a need for further study which will identify the individual compounds that appear at these peaks.

Discussion and Conclusions

In general the patterns of all the chromatograms (Figures 2 to 13) of the whole muscle tissues and the water soluble portions from the mild or strong venison and the beef were similar. It should be emphasized that the interpretation of the findings from the chromatograms of this study was empirical. The fact that when peak 2 was used as a reference point, the time intervals between the peaks Insert
X

Table 15. Peak areas for volatile components from chromatograms of the cutaneous glands of deer

Animal number	Gland	Peak values in square centimeters						
		1	2	3	4	5	6	7
3	Metatarsal	6.04	2.20	0.27	0.05	0.40	----	2.95
3	Tarsal	6.31	1.40	0.18	0.05	0.23	0.49	1.43

were almost identical, ^(1 minute) gave strong evidence ^{omit} that each peak area represented a measure of quantity for a component similar in each sample. Hence, the volatile components of the lean muscle of beef and venison would appear to be identical. The results obtained from the different beef samples were more consistent, whereas in the venison there was quite a variation from animal to animal. There could be many possible causes for this, such as, age and sex of the animal, location and type of range, and season of harvest. Further work on more animals is indicated to obtain more information on this problem.

Strong venison, as defined and used in this study, did show a difference in concentration of components in some of the peak areas (Figures 3 and 6). Analysis of meat with a real strong venison flavor is needed to confirm this finding. Then identification, chemically, of the components would be the next step in pursuing this study.

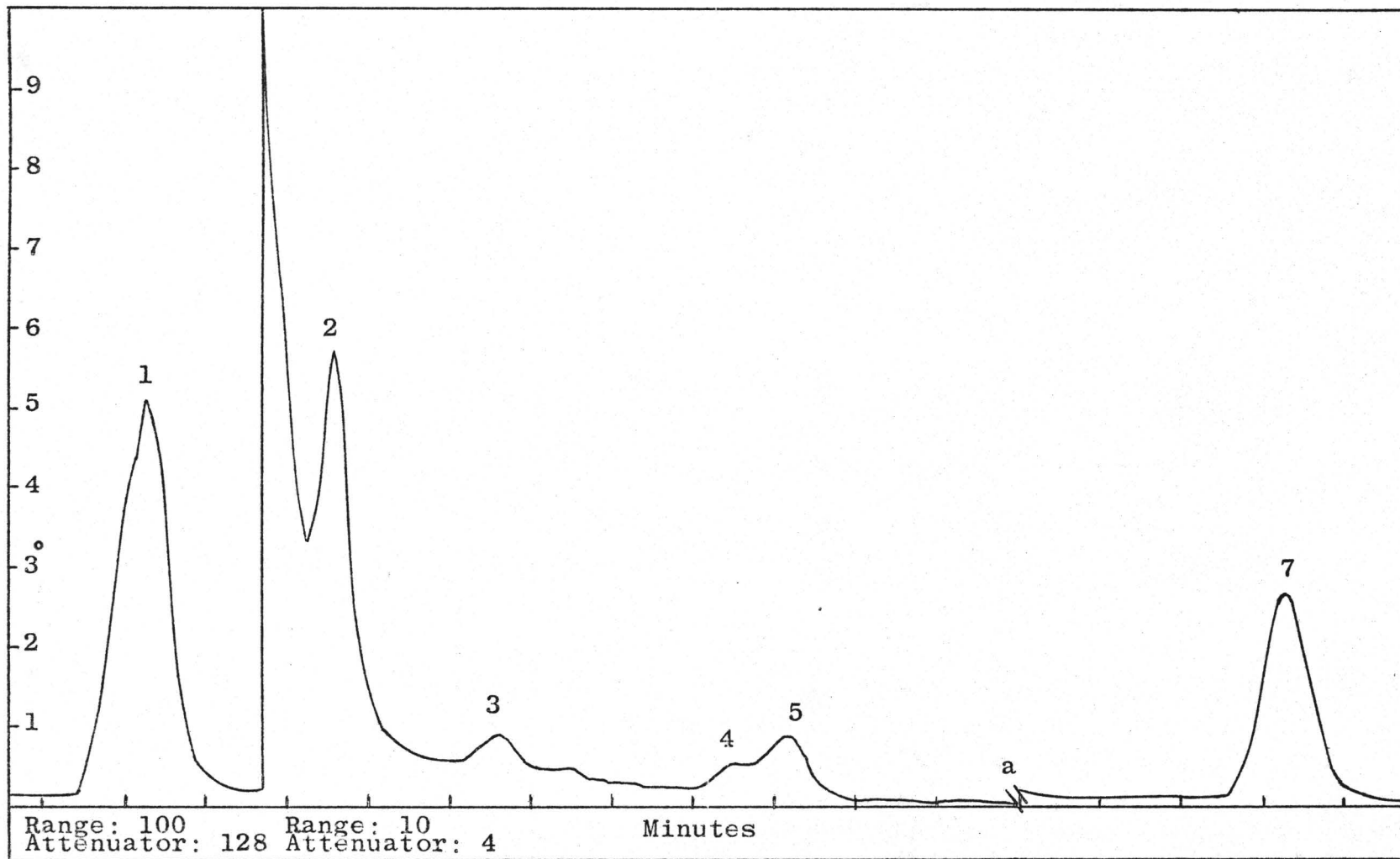


Figure 17. Gas chromatograms of volatile compounds isolated from whole metatarsal gland of venison sample No. 3

^aFold equivalent to 4 minutes run.

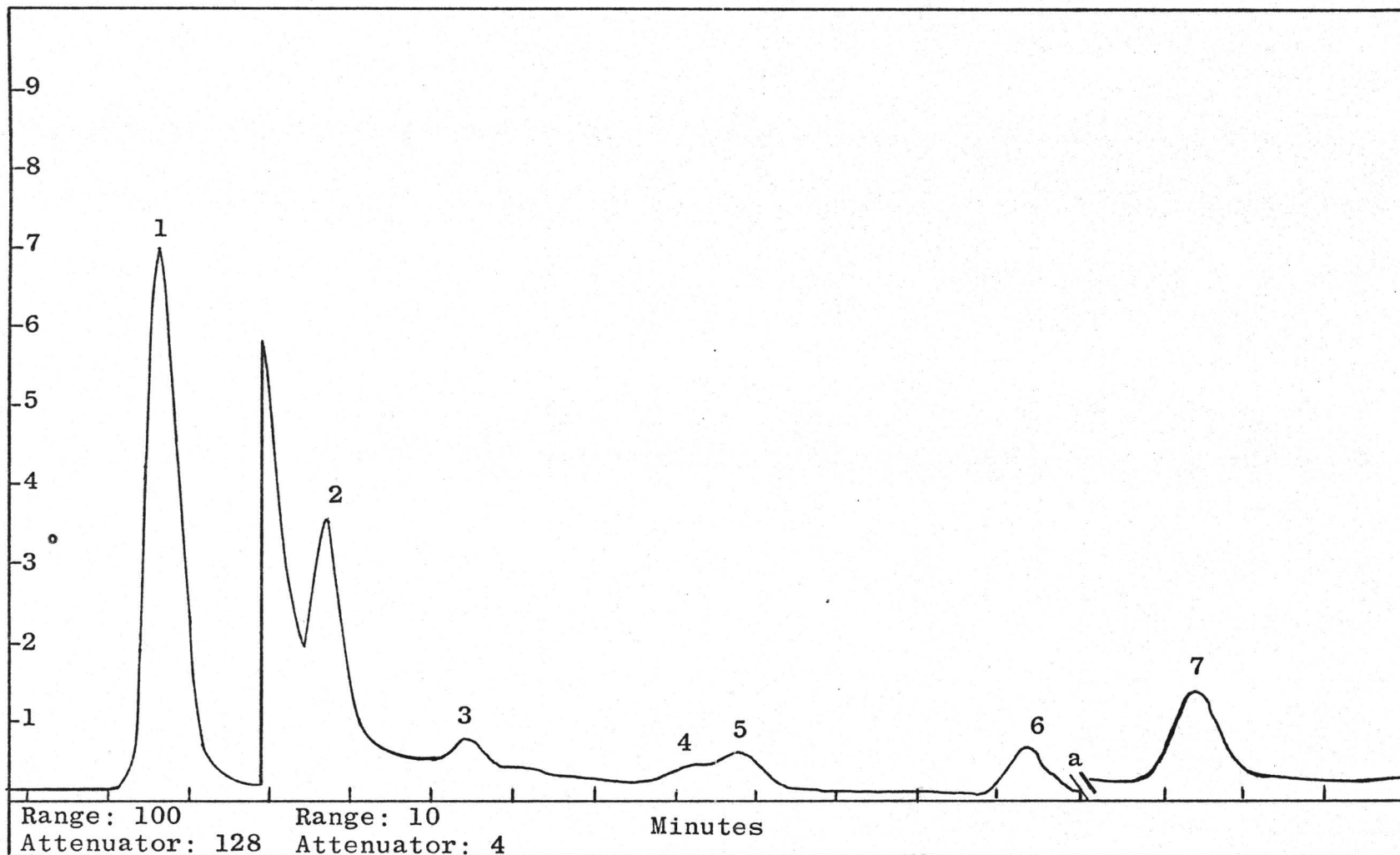


Figure 18. Gas chromatogram of volatile components isolated from whole tarsal gland of venison sample No. 3

^aFold equivalent to 4 minutes run.

In the chromatograms of the ether extracts of the lyophilized samples of lean muscle (Figures 14 to 16) two additional peaks were observed which occurred before peaks 1 and 2, respectively. This did not occur in the chromatograms of either the muscle tissue or water soluble portion. The greatest difference in the concentration or kind of components between the mild and strong venison appeared in peak B. The flavor differences that exist between mild and strong venison may be related to the components occurring at peak B.

Chromatograms of the metatarsal and tarsal glands (Figures 17 and 18) were basically similar in pattern with those of whole muscle tissue chromatograms of venison and beef. The only exception was peak 1 in which the peak area for the glands was represented in greater concentration and/or kind of components than for the muscle. Hence, a definite conclusion cannot be made as to the influence of the strong glandular secretion of these glands on the muscle tissues.

Results obtained from this study indicate that the identification of the flavor components of venison is a highly complex problem. Although many similarities and some possible differences were indicated among the volatile components from mild venison, strong venison and beef, it can be concluded that the components responsible for venison flavor are still to be found.

Possible approaches that could be made for further research on this problem are as follows:

1. The technique as used in this study needs to be refined.
2. More detailed study of ether soluble portions of lyophilized muscle tissue.
3. A detailed study on the cutaneous glands starting with a histological and physiological study of them.
4. Identification of quantity and kind of components emerging at each peak.

Table 16. Time interval between peaks of chromatograms from ether soluble portions and cutaneous glands of venison when peak 2 was used as the reference point

Animal number		Time interval in minutes between peaks					
		2-3	3-4	4-5	5-6	6-7	2-7
		<u>Ether soluble portion</u>					
A	Minutes	2.15	2.75	0.71	-----	10.20 ^a	15.81
	Per cent	13.60	17.39	4.49	-----	64.52	
C	Minutes	1.90	2.60	0.71	-----	10.60 ^a	15.81
	Per cent	12.02	16.45	4.49	-----	67.05	
9 (rt.)	Minutes	2.00	2.65	0.71	-----	10.25 ^a	15.61
	Per cent	12.81	16.98	4.55	-----	65.66	
3 (lt.)	Minutes	1.89	2.75	0.71	-----	10.32 ^a	15.67
	Per cent	12.61	17.55	4.53	-----	65.86	
Mean	Minutes	1.99	2.69	0.71	-----	10.34 ^a	15.73
	Per cent	12.65	17.10	4.51	-----	65.73	
		<u>Cutaneous glands</u>					
Meta-tarsal	Minutes	2.10	2.80	0.71	-----	10.20 ^a	15.81
	Per cent	13.28	17.71	4.49	-----	64.52	
Tarsal	Minutes	1.80	2.65	0.71	3.55	7.10	15.81
	Per cent	11.39	16.76	4.49	22.45	44.91	
Mean	Minutes	1.95	2.73	0.71	-----	10.43 ^a	15.82
	Per cent	12.33	17.26	4.49	-----	65.93	

^aTime interval between peaks 5 and 7.

SUMMARY

Investigations of the causative factors that might influence the flavor and/or odor of venison and beef were made and the results evaluated.

The results of the judges sensory evaluation indicated that the odor was slightly stronger in the ether extract or fat obtained from the lean muscle. However, a considerable portion of the flavor components were detectable in the water extract.

The gas chromatograms, from all the samples, both beef and venison, showed 7 distinct peaks. The areas of these peaks were calculated and compared. Also the general pattern of the chromatograms were compared. Basically both the patterns and peak areas were more or less similar. However, since no attempt was made to chemically identify the peaks, a definite conclusion could not be drawn.

A definite difference was noticed between chromatograms from the whole muscle tissue and the water soluble portion as compared to those of the ether extract of the same muscle tissue. This ^{Copy F} findings ^{in Experiment 1} indicated that the flavor difference in beef and venison could possible be in the fat ^{Insert VI} portion of the lean muscle. ^{to here}

Volatile components from the cutaneous glands in the venison showed similar chromatograms when compared to the muscle tissue chromatograms. Until the peaks are identified

chemically, it will not be possible to draw any conclusions as to possible relationship.

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