Effect of Cellulose Casing on Absorption of Polycyclic Hydrocarbons in Wood Smoke by Absorbents

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Cellulose casing links containing either water or frankfurter emulsion were subjected to various wood smoke conditions. By means of thin-layer silica gel chromatography, fluorescent spots similar in R_f values to anthracene, pyrene, benzo(*a*)pyrene, and 3-methylcholanthrene were found in washings of the outside casing wall. Pyrene, fluoranthene, and anthracene were confirmed by ultraviolet spectrophotometric and fluorometric methods. None of the polycyclic hydrocarbons was found in the absorbents. Quantitative determinatons of benzo(*a*)pyrene in frankfurters following another extraction procedure and cellulose acetate thin-layer chromatography indicated that cellulose casing functioned as a selective barrier to the passage of this smoke component. By contrast, frankfurters smoked in sheep casings contained significantly higher levels of benzo(*a*)pyrene.

The presence of polycyclic hydrocarbons in smoked food products has been detected by several European smoke investigators (Dungal, 1959; Kurko, 1963; Miler, 1962). Some of these hydrocarbons produce a higher than normal incidence of malignant tumors in laboratory animals, and have been classified as carcinogenic substances. The most carcinogenic among these substances are, in decreasing order of their carcinogenicity (Loeb, 1963): 7,12-dimethyl benz(*a*)-anthracene, 7,8,12-trimethyl benz(*a*)anthracene, 3-methylcholanthrene, benzo(*a*)pyrene, and dibenz(*a,h*)anthracene.

Kurko (1963) reported that in work carried out at the Leningrad Institute of Oncology, 7 to 50 μg. of benzo(*a*)pyrene were found per kilogram of smoked fish. Dungal (1959) detected considerable amounts of benzo(*a*)pyrene in lamb and mutton that have been smoked for weeks or months, as is frequently the smoking procedure in Iceland.

On the other hand, American smoke investigators could not detect a similar level of benzo(*a*)pyrene in smoked products available in this country. Porter (1963) using temperatures as high as 500° C. (1022° F.) for wood smoke generation could not find any detectable amounts of benzo(*a*)pyrene in smoke and in smoked cheese. Genest and Smith (1964) recovered 0.01 to 0.05 p.p.m. of standard benzo(*a*)pyrene added to unsmoked food, but no smoked food products examined by them contained this level of benzo(*a*)pyrene. Howard *et al.* (1966a) found 3.2 p.p.b. in smoked ham, and smaller amounts in smoked sturgeon and dried smoked herring. Malanoski and coworkers (1968) found 0.5 to 0.7 p.p.b. of polycyclic hydrocarbons in a variety of smoked foods. Lijinsky and Shubik (1965) found 1 p.p.b. or less in smoked fish and 6 p.p.b. in charcoal broiled steaks and 10 p.p.b. in barbecued ribs. A benzo(*a*)pyrene content as high as 50 p.p.b. was found in one instance in charcoal broiled steak (Lijinsky and Ross, 1967). Apparently, the general degree of smoking in European countries is somewhat in excess of the smoking practices on this continent (Wilson, 1961).

Smoke generation temperature was a decisive factor influencing the generation of these polycyclic hydrocarbons. Miler (1962) and Tilgner (1965) reported that carcinogens are not generated when the temperature in the wood destruction zone does not exceed 425° C. (approximately 800° F.) and 375° C. (approximately 700° F.) in the oxidation zone. To avoid formation of benzo(*a*)pyrene, Kurko (1963) suggested controlling the combustion temperature of sawdust and keeping it below 300° C. (572° F.). Dikun *et al.* (1966) found benzo(*a*)pyrene and other polycyclic hydrocarbons at temperatures less than or equal to 300° C. and that more benzo(*a*)pyrene was produced from softwoods than hardwoods. Rhee and Bratzler (1968) separated and identified 11 polycyclic hydrocarbons, which included benzo(*a*)pyrene, from hard maple sawdust smoke generated at smouldering temperature of 750° to 800° C.

The purpose of this investigation was to study the effect of cellulose casings on the absorption of polycyclic hydrocarbons in wood smoke during sausage processing.

EXPERIMENTAL

Smoke Generator. Experiments were performed with an electrically heated smoke generator in which the wood source was kiln-dried maple sawdust (Simon *et al.*, 1966). The sawdust feed was set to deliver 25 pounds of sawdust per hour; the smoke generator was operated at 575°, 650°, 700°, or 750° F. This range was selected because only small amounts of sawdust would burn below 575° F., and the generator was not equipped to operate at 780° F. or above.

Absorbents. WATER LINKS. Nojax casing, size 24 (Food Products Division, Union Carbide Corp.), was used to prepare the water link absorbents. The casings were washed to remove glycerine and cut into approximately 30-cm. lengths. One end was knotted and 50 ml. of distilled water were added. The open end was then twisted at the top of the water level until the diameter of the tube reached 22-mm., the link diameter recommended by the manufacturer. At this point the casing was again knotted.

FRANKFURTERS. The frankfurters were prepared from meat emulsion made according to customary commercial comminuting practices from ingredients listed in Table I. The emulsion was stuffed into size 24 Nojax casings and the frankfurters linked at 13-cm. lengths to 22-mm. diam. In most instances, the frankfurters were subjected to the same smoke environments as the water links.

For benzo(*a*)pyrene determinations frankfurters were processed in sheep casings for comparison with those prepared in cellulose casings.

Smokehouse Process. The absorbents were suspended in an air conditioned one-cage smokehouse (Simon *et al.*, 1966). With the smokehouse and smoke generator used, the smoke

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Table I. Frankfurter Formulation

Boneless chucks	25 lb.
Regular pork trimmings	25 lb.
Ice	15 lb.
Salt	1.5 lb.
Seasoning[a]	6.0 oz.
Cure[b]	2.0 oz.
Sodium iso-ascorbate	12.5 g.

[a] Commercial frankfurter seasoning.
[b] Commercial mixed cure containing $NaNO_3$ and $NaNO_2$ to extent that 1/4 oz. $NaNO_2$ was used per cwt. meat.

exposure was 3 to 5 minutes for commercially acceptable frankfurters. To obtain a high yield of smoke constituents for silica gel thin-layer chromatography (TLC) study, the links were subjected to 30 minutes smoke at house temperatures of 130°, 180°, and 250° F., and 10, 25, or 40% RH. These temperatures and humidities correspond with oven conditions encountered in commercial frankfurter manufacturing operations in the United States.

For benzo(*a*)pyrene determinations, the frankfurters were smoked at about a 700° F. generator temperature for 0, 5, or 15 minutes and cooked in accordance with commercial practice at 140° to 180° F. (40% RH) for 30 minutes. The product was then held at 180° F. for about 10 minutes until internal temperature reached 160° F. Half of these processed samples were peeled the next day and the other half of the samples were stored in the Nojax casing for 1 week in a 30° F. cooler.

Silica Gel TLC. Genest and Smith (1964) used benzo(*a*)pyrene as an index of smoke carcinogens. They developed a silica gel TLC method for the detection of 0.01 to 0.05 p.p.m. benzo(*a*)pyrene in foods. For the present investigation, in addition to benzo(*a*)pyrene, other polycyclic hydrocarbons were selected for investigation on the basis of reported carcinogenic activity (Eckardt, 1959) and/or availability of standards. These included anthracene, dibenz(*a*,*h*)anthracene, pyrene, naphthacene, and 3-methylcholanthrene. All compounds were obtained from Aldrich Chemical Co., Inc., with the exception of 3-methylcholanthrene which was purchased from Eastman Kodak Co. Standards were prepared with these compounds by dissolving 1 mg. of each in 1 liter of 2,2,4-trimethylpentane. An aliquot of 0.01 ml. of each standard was spotted on a plate alongside the unknown extracts and the R_f and fluorescent color of the spots compared under ultraviolet irradiation. The purity of the standards was checked by TLC procedures. The low solubility of naphthacene in the solvent and the lack of migration with the developer solvent prevented suitable means for detecting this compound by TLC.

The procedure of Genest and Smith (1964) was used in detail for extraction of polycyclic hydrocarbons from frankfurters. Some minor changes were made to collect the polycyclic hydrocarbons from the casing and water links. A 100-ml. sample of smoked water (from water links) and smoke covered cellulose casings from three links were used for separate analysis. The smoke components from these materials were extracted directly with benzene, dried by passage through an anhydrous sodium sulfate bed, and condensed under nitrogen gas to 1 ml. on a steam bath.

A 50-μl. portion of concentrate from inside the casing and a 25-μl. portion from the concentrated washings of the outside surface of the casing were spotted on a 0.75-mm. silica gel G covered glass plate (Brinkmann, New York). After developing three consecutive times with a mixture of 97% 2,2,4-trimethylpentane and 3% benzene, the plates were examined under a 2537A. and 3660A. ultraviolet lamp (Ultra-Violet Products, Inc., San Gabriel, Calif.) for fluorescence, the intensity of which was determined visually. The 2537A. (short wave) lamp was used in addition to the 3660A. (long wave) lamp used by Genest and Smith (1964) as anthracene fluoresced under the short wave lamp, but not under the long wave lamp.

The spots were eluted from the silica gel with 2,2,4-trimethylpentane and concentrated to 0.5-ml. volume. The ultraviolet absorption spectra were determined on a Cary 14 recording spectrophotometer (Applied Physics Corp., Monrovia, Calif.) using fused quartz cells of 0.5-ml. capacity and beam masks. Fluorescent emission spectra were obtained with an Aminco-Bowman spectrophotofluorometer (American Instrument Company, Inc., Silver Spring, Md.). The instrument was equipped with an ellipsoidal mirror xenon lamp housing and variable slits on the lamp phototube compartment and cell holder. The slit system used throughout this work was as follows: lamp and phototube, 1 mm.; monochromators, 3 mm.; cell holder slits, 1.5 mm.; and light scatter slits, 3 mm.

Benzo(*a*)pyrene Determination. Benzo(*a*)pyrene values were quantitatively determined in frankfurters using the extraction and isolation techniques described by Howard *et al.* (1966a) with the elimination of the paper chromatography step as reported by Howard (1966b). By this procedure, 85 to 100% recovery of benzo(*a*)pyrene was obtained at a level of 3 and 5 p.p.b. from a fortified 500-gram nonsmoked frankfurter sample. To ensure thorough soxhlet extraction of the sample by ethanol, a perforated tube as described in the procedure (Howard *et al.*, 1966a) for cheese extractions, was used for the frankfurters. All solvents and standards were purified and tested for purity in accordance with the procedure (Howard *et al.*, 1966a). To compare R_f values and locate benzo(*a*)pyrene in the samples, standard benzo(*a*)pyrene was chromatographed on the thin-layer acetylated cellulose plates alongside each unknown. The procedure of Howard *et al.* (1966b) was subjected to a collaborative study (Howard *et al.*, 1968) on the recovery of benzo(*a*)pyrene from fortified fish and ham samples with resultant high levels of recovery and precision from samples fortified with 4 and 10 p.p.b. benzo(*a*)pyrene.

An Aminco-Bowman spectrophotofluorometer was used to obtain benzo(*a*)pyrene values and spectra. Emission spectra were obtained using an excitation wavelength of 385 mμ. Quantitative fluorometric measurements were made using an excitation wavelength of 385 mμ and an emission wavelength of 408 mμ. Detection limit for benzo(*a*)pyrene with this instrumental arrangement was about 0.01 μg. per ml. of solution. Statistical significance was determined using Student's *t*-test (Snedecor, 1956).

After separation of the purified benzo(*a*)pyrene fraction by TLC, ultraviolet absorption spectra were obtained with a Cary Model 14 spectrophotometer. The spectra were used for confirmation of benzo(*a*)pyrene and not for quantitative analysis.

RESULTS AND DISCUSSION

Absorbents. The concentrates from washing the outside surface of casings left a long path of brilliant fluorescence with some more or less defined spots (Figure 1) on the silica gel plates. With three or four fluorescing spots, the total fluorescence was assessed as +++++ or ++++; with two spots, +++ or ++; and with one spot, + or trace. In general, the number of these spots was proportional to the total intensity of fluorescence.

The determination of polycyclic hydrocarbons of smoke

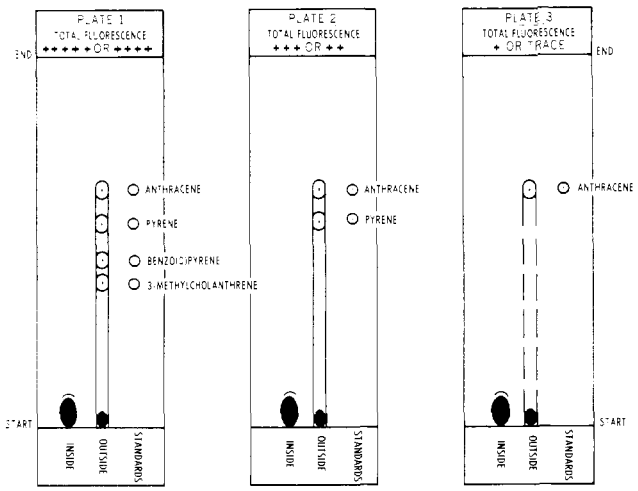


Figure 1. Chromatographic plates of smoke samples from outside and inside the water link absorbents

Three plates were prepared from samples obtained from three different smoke exposures. See footnote Table III for explanation

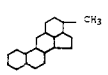
deposited on the outside surface of casings or the contents of the absorbents was limited to a qualitative procedure because of the nonsuitability of the silica gel technique for quantitative analysis (Genest and Smith, 1964).

All spots were compared with known standards regarding their R_f values and fluorescent color. In this way, fluorescent spots with R_f values equivalent to anthracene, pyrene, benzo(*a*)pyrene, and 3-methylcholanthrene were found in the concentrates from the outside casing wall (Table II). Of these, anthracene appeared as a well defined spot. The benzo(*a*)pyrene spot was not always too clearly defined and its presence was sometimes questionable. No fluorescent spots were found to compare with the standard of dibenz(*a,h*)anthracene.

The concentrates from inside the casing, when spotted on a plate, only showed a slight fluorescence in form of a faint halo above the point of introduction (Figure 1). This was true of the water link samples as well as the frankfurter extracts.

Polycyclic hydrocarbons were found only on the outside surface of the casing. Not a single case was encountered in which the known polycyclic hydrocarbons penetrated the casing into water or meat. Under conditions exercised, none of the fluorescing hydrocarbons diffused through the casing.

Table II. Fluorescent Color and Sequence of Polycyclic Hydrocarbons Chromatographed on a Silica-gel G Plate

Compound	Structure	Spot Color
Anthracene ($C_{14}H_{10}$)		Violet
Pyrene ($C_{16}H_{10}$)		Blue
Fluoranthene ($C_{16}H_{10}$)		White
Benzo(*a*)pyrene ($C_{20}H_{12}$)		Navy blue
3-Methylcholanthrene ($C_{21}H_{16}$)		Light blue

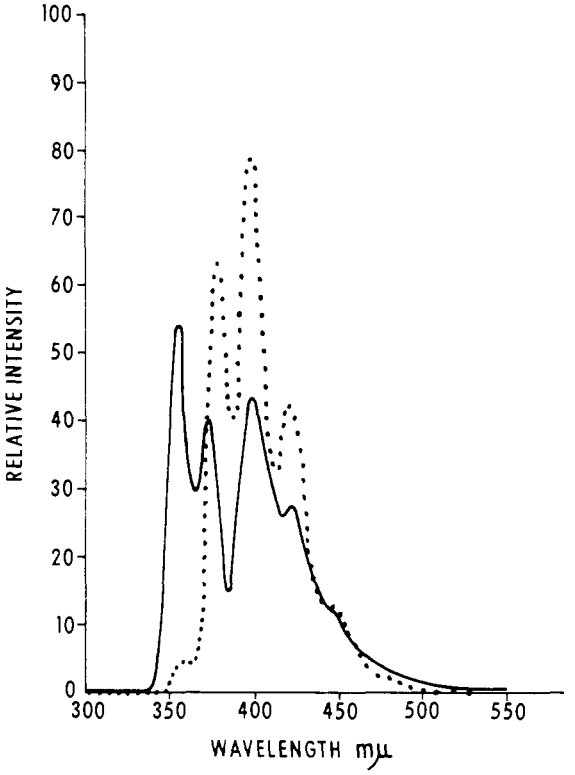


Figure 2. Fluorescence emission spectrum at excitation 355 mμ of polycyclic hydrocarbons eluted from spot 1 of chromatogram of outside casing wall extracts

Solid line, unknown, 0.001 mm.; broken line, anthracene reference curve, 0.001 mg. per ml. in 2,2,4-trimethyl pentane, 0.01 mm.

No significant differences were observed between the use of a water link and a meat link with respect to polycyclic hydrocarbon accumulation. At 250° F. house temperature, however, frankfurter emulsion breakdown frequently occurred and the release of fat droplets onto the outside surface of a meat link was experienced during casing removal. The fat apparently washed away some of the smoke components as the total fluorescence of these concentrates was markedly lower than those of the corresponding water links.

Under the conditions tested, it may be concluded that although polycyclic hydrocarbons were generated during the wood smoking process, the cellulose casing was an effective barrier in preventing transfer to the absorbents. The commercial practice relative to rapid manufacture of skinless frankfurters whereby casing removal occurs shortly after processing and prior to packaging would thereby leave the product free from these undesirable smoke constituents. Howard *et al.* (1966a) found fluoranthene and pyrene in the parts per billion range in smoked frankfurters, but did not indicate the source of the frankfurters or the manufacturing conditions.

Spectrophotometric Investigation. The fluorescent spots which appeared on the chromatograms from the outside casing wall extracts were eluted for spectrophotometric confirmation. The ultraviolet absorption spectra of eluted Spots 1 and 2 (with R_f values equivalent to anthracene and pyrene, respectively) were recorded. The major absorption peak of anthracene at 253 mμ and the minor peaks at 358 and 376 mμ appeared in the spectrum of Spot 1. Other absorption peaks which corresponded to those of pyrene and fluoranthene appeared also, and were indicative of the impurity of the sample. Attempts to purify the sample of Spot 1 by re-

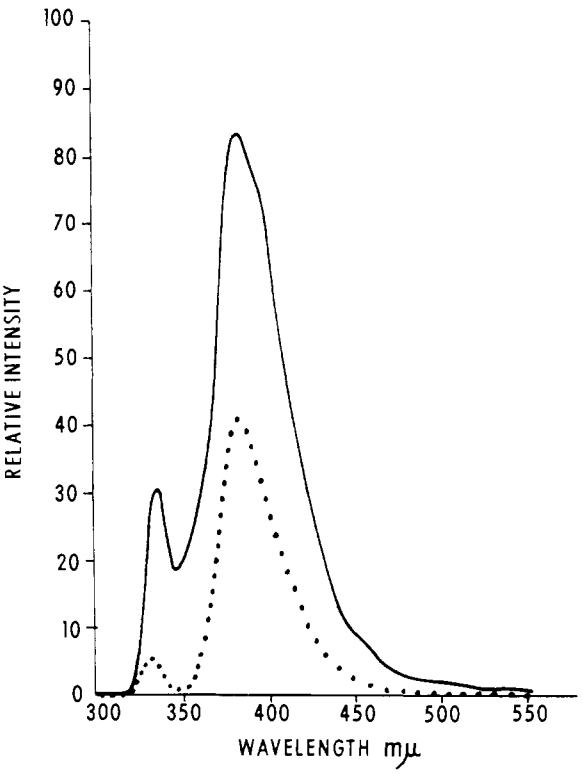


Figure 3. Fluorescence emission spectrum at excitation 333 mμ of polycyclic hydrocarbons eluted from spot 1 of chromatogram of outside casing wall extracts

Solid line, unknown, 0.001 mm; broken line, pyrene reference curve, 0.001 mg. per ml. in 2,2,4-trimethyl pentane. 0.003 mm.

chromatograming were unsuccessful in separating the components and lead only to a loss of sample as expressed by an attenuation of all absorption peaks. A fluorescent emission spectrum of Spot 1 (Figure 2) confirmed the presence of anthracene. An emission spectrum of pyrene (Figure 3) was observed from the same spot.

Eluted Spot 2 was rechromatogramed and separated into two separate fluorescent spots, one trailing just behind the other. The spectrum of the leading spot appears in Figure 4 and of the trailing spot in Figure 5. The major absorption peaks of pyrene (Figure 4) and fluoranthene (Figure 5)

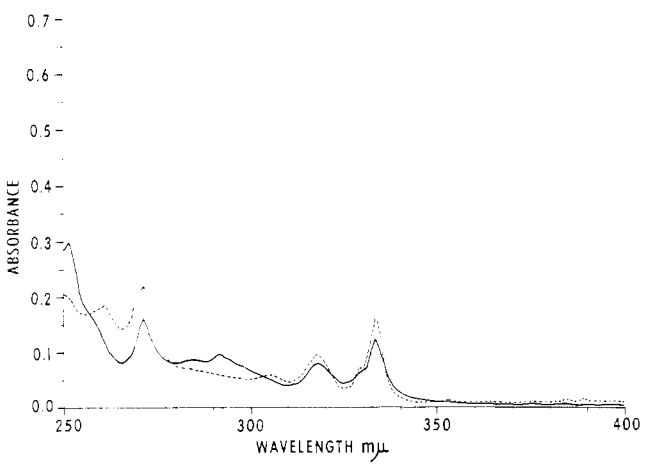


Figure 4. Ultraviolet absorption spectrum of blue fluorescent spot (solid line) obtained after rechromatogram of spot 2

Broken line, pyrene reference curve (0.0005 mg. per ml. in 2,2,4-trimethyl pentane)

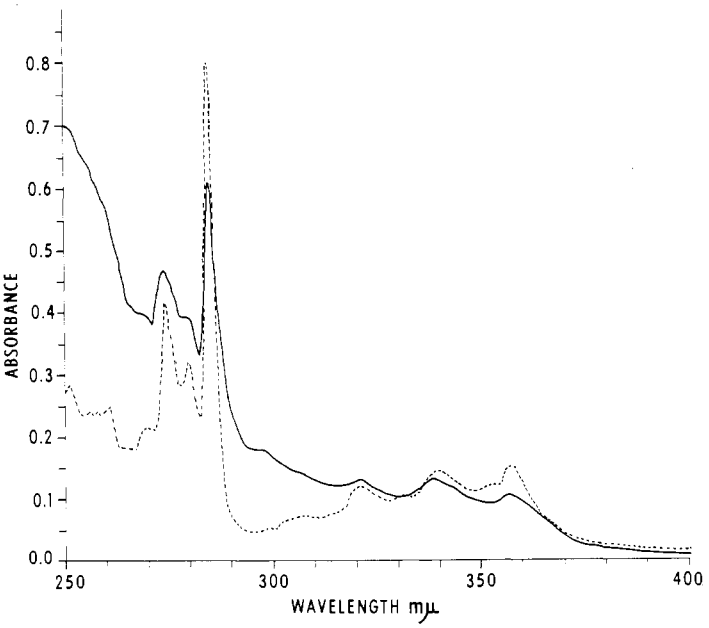


Figure 5. Ultraviolet absorption spectrum of white fluorescent spot (solid line) obtained after rechromatogram of spot 2

Broken line, fluoranthene reference curve (0.001 mg. per ml. in 2,2,4-trimethyl pentane)

appear in each spectrum. An attenuation of the absorption band of the fluoranthene sample spectrum at about 298 mμ was accomplished by addition to the reference cell of fractional quantities of benzo (*g,h,i*)perylene (about 0.43 μg. per ml.), which has a strong absorption peak at this wavelength. The presence of benzo(*g,h,i*)perylene on the smoked absorbents was not further indicated. An emission spectrum of fluoranthene was obtained from Spot 2 before it was rechromatogramed (Figure 6). Benzene extracts of unsmoked casings were chromatogramed and the fluorescent regions corresponding to the smoked sample spots were eluted. Spectra of these spots indicated the presence of an absorption with no characteristic peaks, but with increasing absorption from about 310 to 250 mμ. This may account, in part, for the overall rise noted in this region of the sample spectra and account for the background interferences observed. Eluted Spot 3 [R_f value equivalent to benzo(*a*)pyrene] and Spot 4 (R_f value equivalent to 3-methylcholanthrene) showed no major peaks in the ultraviolet absorption or emission fluorescence spectra corresponding to the known compounds, but indicated contamination with other substances (*i.e.*, pyrene, fluoranthene). Attempts to purify these spots by rechromatographing on silica gel were not fruitful.

Smokehouse and Generator. The data in Table III indicate that the temperature of the smokehouse had a pronounced effect on the concentration of the fluorescing hydrocarbons. At 130° F. smokehouse temperature, only the fluorescent anthracene spot could be found. At 250° F., as many as four fluorescent spots were found. This may be attributed to the influence of molecular weight on volatility of these compounds at different temperatures. Within the conditions tested, RH and generator temperatures were of little or no significance on production and/or absorption of fluorescent materials on the cellulose casing wall. Since the generator temperatures correspond to that of the bottom plate of the smoke generator and not necessarily to the wood combustion zone, it would be difficult for the authors to assess their findings with those of Kurko (1963), Miler (1962), and Tilgner

Table III. Detection of Polycyclic Hydrocarbons by Silica Gel TLC on Water Link Samples Exposed to Smoke for 30 Minutes

Smokehouse Temp., °F	Generator Temp., ° F	Smokehouse R.H., %		Benzo(*a*)pyrene		Fluorescence Total Unidentified[a]	
				Inside link	Outside link	Inside link	Outside link
	750	10		−	+	sl. halo	+++++
250	650	10		−	Trace	sl. halo	+++++
	575	10		−	—	sl. halo	++++
	750	25	40	−	Trace	sl. halo	+++
180	650	25	40	−	?	sl. halo	++
	575	25		−	—	sl. halo	+
	750		40	−	—	v. sl. halo	+
130	650		40	−	—	v. sl. halo	Trace
	575		40	−	−		−

[a] +++++ or ++++, three or four fluorescing spots clearly distinguished (Plate 1, Figure 1).
+++ or ++, two fluorescing spots clearly distinguished (Plate 2, Figure 1).
− or trace, one fluorescing spot clearly distinguished (Plate 3, Figure 1).
−, no fluorescence.

(1965) in relation to the influence of combustion temperature on formation of polycyclic hydrocarbons.

Benzo(*a*)pyrene Permeation. Benzo(*a*)pyrene also was determined in frankfurters by a more sensitive extraction and detection procedure adapted from Howard *et al.*, (1966a, 1966b) than mentioned above (Genest and Smith, 1964). This compound was selected for closer examination in frankfurters as it is a carcinogenic material for rats (Dungal, 1959; Kurko, 1963; Vigdorovich, 1946). The fluorescent emission spectra obtained for unsmoked skinless and animal casing frankfurters and for samples prepared by smoking for 15 minutes are shown in Figure 7. A benzo(*a*)pyrene reference spectrum was included on each figure for comparison. All samples showed the presence of benzo(*a*)pyrene characteristic fluorescent peaks of 408, 429, and 455 mμ. Some variation in relative intensities was observed from one spectrum to another, which probably was due to extraneous material from the extraction technique at the scale factors used to obtain the spectrum. The relative peak intensities of the benzo(*a*)pyrene extracts of frankfurters processed in animal casings (higher scale factor) more closely simulated that of the benzo(*a*)pyrene standard than that of the skinless frankfurter samples (lower scale factor). The contribution of the background fluorescence was greater for those spectra obtained at lower scale factors (Figure 7, *A* and *C*) than those obtained at higher scale factors (Figure 7, *B* and *D*).

As shown in Table IV, the benzo(*a*)pyrene values determined in skinless frankfurters averaged 1.3 p.p.b. for unsmoked product and 1.6 p.p.b. for smoked product. Solvent and unprocessed meat blanks emitted fluorescence under benzo(*a*)pyrene measuring conditions which resulted in an average value of 0.3 p.p.b. For all processing conditions used, no statistically significant differences existed between the unsmoked skinless frankfurters and those prepared using

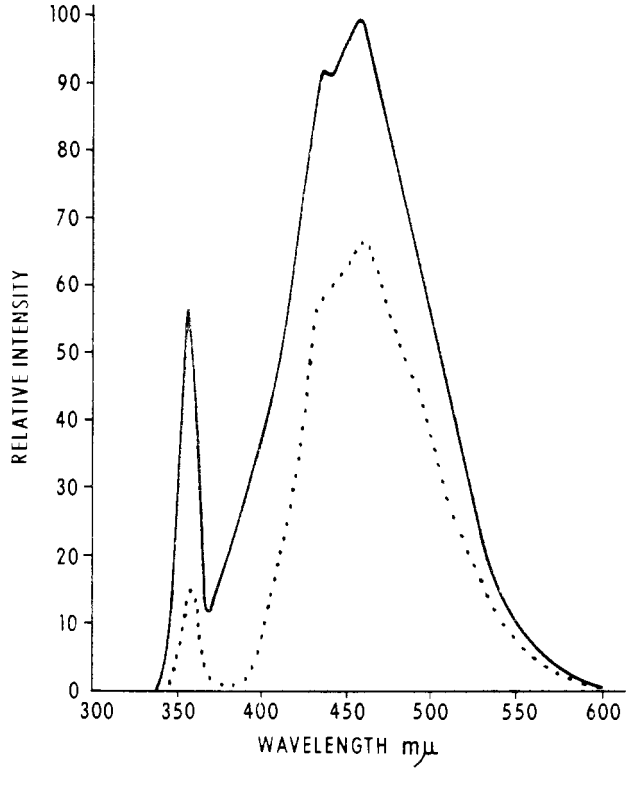


Figure 6. Fluorescence emission spectrum at excitation 357 mμ of polycyclic hydrocarbons eluted from spot 2 of chromatogram of outside casing wall extracts

Solid line, unknown, 0.001 mm.; broken line, fluoranthrene reference curve, 0.001 mg. per ml. in 2,2,4-trimethyl pentane, 0.003 mm.

Table IV. Benzo(*a*)pyrene Values of Frankfurters Processed in Cellulose and Sheep Casings[a]

Sample[b]	Smoking Time, Min. 0	5	15
SKINLESS FRANKFURTERS			
Number of samples	11	8	12
Av. p.p.b.	1.3	1.6	1.6
Standard deviation	1.2	0.4	1.0
ANIMAL CASING FRANKFURTERS			
Number of samples	9	4	4
Av. p.p.b.	2.1	4.0[c]	13.0[c]
Standard deviation	0.8	1.7	1.5
BLANKS			
Number of samples[d]	7		
Av. p.p.b.	0.3		
Standard deviation	0.1		

[a] Frankfurters were smoked for 0, 5, or 15 minutes at a generator temperature setting of about 700° F.
[b] Samples represent 2 to 6 separate processing runs.
[c] $P < 0.02$ by Student's *t*-test.
[d] Included 4 meat and 3 solvent blanks.

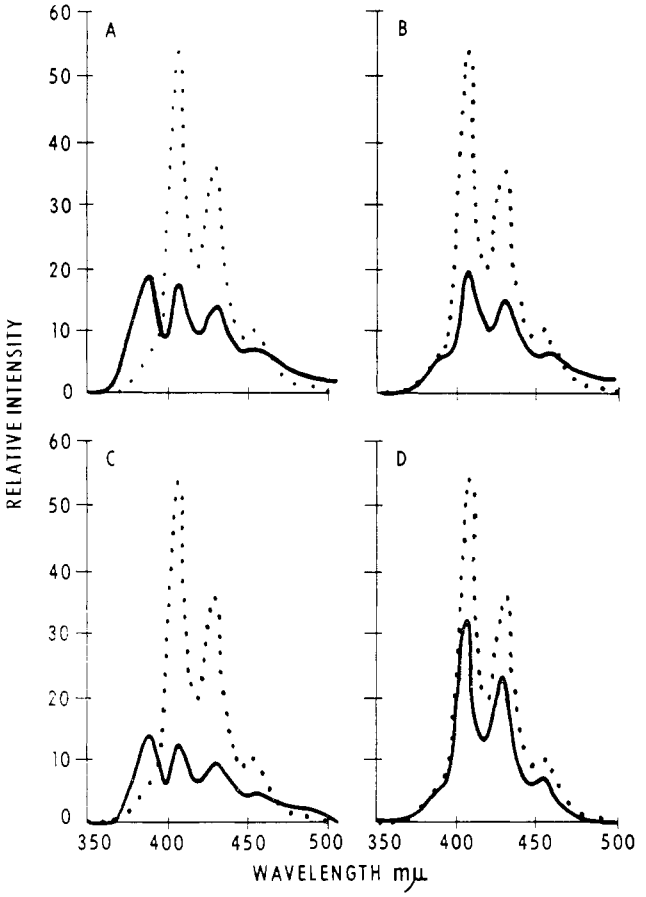


Figure 7. Fluorescence emission spectra at excitation 385 mμ of benzo(*a*)pyrene isolated from frankfurter absorbents

Solid line, unknown; broken line, reference standard 0.001 mg. per ml., 0.1 mm. *A*. Skinless, no smoke, 0.01 mm.; *B*. Sheep casing, no smoke, 0.1 mm.; *C*. Skinless, 15 minutes smoke, 0.03 mm.; *D*. Sheep casing, 15 minutes smoke, 0.1 mm.

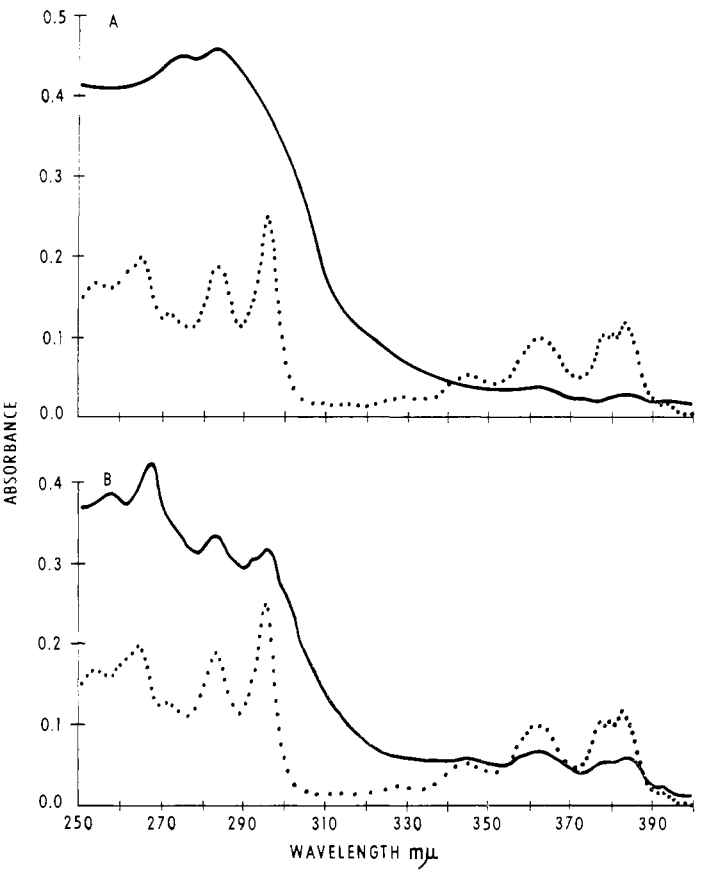


Figure 8. Ultraviolet absorption spectra of benzo(*a*)pyrene isolated from frankfurter absorbents

Solid line, unknown; broken line, reference standard 0.001 mg. per ml. (*A*). Sheep casing, no smoke; (*B*). Sheep casing, 15 minutes smoke

either 5 or 15 minute smoking periods. Malanoski *et al.* (1968) reported levels of less than 0.5 and 0.8 p.p.b. benzo(*a*)-pyrene in two frankfurter samples and could not detect benzo-(*a*)pyrene in three other frankfurter samples. Gunther and Buzzetti (1964) reviewed the work of others discussing formation of benzo(*a*)pyrene in heated, but nonsmoked foodstuffs—*i.e.*, roasted coffee beans, bread, and starch. Whether or not this situation existed in the skinless frankfurter processing or the benzo(*a*)pyrene values merely reflected blank values as a result of sample and method interaction, remains to be clarified. No differences in benzo(*a*)pyrene values were found in frankfurter samples stored in the cellulose casings one week prior to casing removal, (exaggerated commercial practice) and those peeled under normal commercial practices (immediate casing removal or after overnight storage in a cooler).

There was a significant increase in the benzo(*a*)pyrene values in smoked animal casing frankfurters compared with unsmoked product. The fluorescent spectra of these samples (Figure 7, *D*) were similar to that of a standard benzo(*a*)-pyrene solution, and the presence of benzo(*a*)pyrene was further confirmed by the similarity of the absorption peaks in the ultraviolet spectra of the sample to that of standard benzo(*a*)pyrene solution (Figure 8). The presence of benzo-(*a*)pyrene in the 5- and 15-minute smoked animal casing frankfurters as a result of the smoking process indicated the presence of benzo(*a*)pyrene in the smoke.

The cellulose casings acted as a barrier to benzo(*a*)pyrene which prevented permeation of this polycyclic hydrocarbon during smoking of the sausage. In this regard, the advantage of cellulose casing over animal casing is apparent. The casing does permit compounds to permeate which contribute to smoked flavor and color (Simon *et al.*, 1966). Others (Dobes *et al.*, 1954; Gorelova and Dikun, 1958a,b; Tilgner *et al.*, 1958) have shown that benzo(*a*)pyrene migrated through the skin and penetrated into the meat of various smoked fish and sausage. Dense fish scales reduced benzo(*a*)pyrene penetration (Gorelova and Dikun, 1958a). In the case of the sausage (Gorelova and Dikun, 1958b), the casing was not identified by the investigators; however, it is reasonable to assume that it was made from animal intestine, since that was the predominant casing in Russia. If that were the case, then our findings in relation to frankfurters smoked in sheep casing would support the findings of Gorelova and Dikun (1958b), rather than contradict them.

Casing Permeability. Knowledge of the transport process through cellulose membranes is congruous with the test results obtained on barrier characteristics of the casing. Regenerated cellulose film is fundamentally a gel which shares the properties of other gelatinous materials which are hydrophilic (Tuwinner, 1962). Consequently, transport of substances through a cellulose membrane would depend on the solubility relationship between the film and components of the fluid mixture (Kammermeyer, 1956). Gas permeability of cellulose membranes was directly related to the moisture sorbed by the film (Buchner, 1960; Hauser and McLarnen,

1948) and to the solubility of the gas in water (Barrer, 1951).

Since cellulose is hydrophilic, the literature is essentially void of cellulose permeability studies with strictly hydrophobic substances. Aromatic polycyclic hydrocarbons being hydrophobic in nature and insoluble in water (Lange, 1961) would not be expected to permeate cellulose film. The nonpermeable nature of cellulose casing to oil or fat is recognized historically by sausage men in the meat industry experienced with processing in cellulose casing. When fat rendering occurs during heat processing, and even after prolonged storage, the fatty substances are retained inside the link. Tests in this laboratory, whereby the cellulose casing link was filled with lard and held at 212° F. for one week, showed no signs of permeating the membrane. No fat or oil was detected on the outside casing surface. On the other hand, sheep casings, being primarily protein with some fat, will allow hot fat to drain or permeate. A cellulose casing link filled with 50 ml. of benzo(*a*)pyrene in hexane (0.01 mg. per ml.) was dialyzed against 35 ml. of hexane in a 100-ml. capacity cylinder for one week at ambient (73° F.) temperature. During this test, no chromatographic or spectrophotometric evidence was obtained to indicate migration of benzo(*a*)pyrene through the membrane wall.

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Correction

LUMINESCENCE ANALYSIS OF FOOD ANTIOXIDANTS. DETERMINATION OF PROPYL GALLATE IN LARD

In this article by H. W. Latz and R. J. Hurtubise [J. AGR. FOOD CHEM. **17,** 352 (1969)], the captions on Figures 1 and 2 should be reversed.
