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SEPARATION AND IDENTIFICATION OF POLYCYCLIC

AROMATIC HYDROCARBONS IN RUBBER DUST

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SEPARATION AND IDENTIFICATION OF POLYCYCLIC

AROMATIC HYDROCARBONS IN RUBBER DUST

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CHAPTER I

INTRODUCTION

The continually increasing number of motor vehicles is perhaps the most outstanding feature of modern society. Even feudal and backward Spain has begun to spawn motor vehicles in such numbers that the population refers to one popular model as the "belly button" since everybody has one. In America, conservationists are becoming troubled at the quantity of farm land which is being gobbled up by the bulldozer as earth is removed and long ribbons of concrete excreted to provide roads that are overcrowded from the day they are opened.

Greater numbers of automobiles mean not only greater quantities of exhaust fumes which have become exceedingly troublesome in the atmosphere of metropolitan areas but also larger and larger quantities of rubber which have been abraded into both air and soil. This latter source of pollution would probably be a negligible health hazard except for the presence in vehicle tire rubber of a vector for a group of organic compounds belonging to the polycyclic aromatic group which enter the rubber through their adhesion to carbon black used as a filler. Some of the compounds are carcinogens, and, though they are generally considered

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inert in rubber, they can, under certain circumstances, be a hazard to health. Investigations reporting the presence of polycyclic aromatics in the waters of lakes and rivers suggest the possibility that as the rubber has aged and abraded these compounds have been released to accumulate in soil and water. The presence of these products further suggests the need for analytical techniques for following their formation, accumulation and degradation in the environment.

CHAPTER II

LITERATURE REVIEW

Possibly the earliest indictment of carbonaceous products as a cause of disease was drawn by Percival Pott in 1775 (1). His investigation of soot as a cause of scrotal cancer in chimney sweeps was a classical study in occupational cancer and has led to greater advances in the knowledge of the causation and prevention of tumor formation than any other line of inquiry.

Paget (2) gave this description:

> The skin of the exposed parts of the body as well as of the scrotum which are more or less covered with soot becomes dry and seborrheic. Soot outlines the fine folds of the skin and is present particularly in the depths of the rugae of the scrotal sac where black plugs obliterate the openings of the excretory ducts of the sebaceous glands. These changes are followed by the development of small, but usually numerous, warts which may cover densely the genital region.

Further appropriate comment is from Curling, quoted by Hueper (3):

> These papillomatous formations may persist for months or even years without undergoing any further changes. However, some may become fixed at their base in the course of time and may ulcerate later. The edges of these ulcerated epitheliomatous defects are elevated, firm and indurated, while a fetid, serous material exudes from the ulcerated surface. The ulcer and the surrounding indurated area gradually spread, often form vegetative excrescences, and may finally involve the testes, spermatic cord, penis, large femoral vessels and inguinal nodes. The cancerous growth may ultimately penetrate into the abdominal cavity and affect adjacent organs. Metastases into the remote organs are rare. Recurrences at other parts of the scrotal sac after local removal of the first growth are evidently frequent, especially if hazardous work is continued.

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Second primary tumors may appear many years after the cessation of the exposure to soot. The histologic examination of the cancerous lesions in the skin of chimney sweeps showed in every case the presence of cornified squamous cell carcinomatous structures exhibiting a low degree of proliferative activity.

These observations of more than 100 years ago, while their discussion of cause should be more circumspect, posed a target at which subsequent investigators have struck telling blows. Though the exact mechanism of inducing cancer still may be concealed, protection from a dread disease is possible, indeed obligatory, by hygienic measures reducing or preventing contact with the offending agent. These early observations and descriptions of an occupational association speeded up legislation for the protection of the population at risk in exposed situations.

The sufferings of victims of occupational disease initiated the attack on the problem of the cancer which is assumed to be caused, in part at least, by exposure to a specific substance. In 1892 Butlin (4) showed that workers developing scrotal cancer had an occupational exposure to pitch, tar and mineral oil. In 1915 Yamagiwa and Ichikawa (5) produced cancer experimentally by painting the ears of rabbits with coal tar, and in 1922 Leitch (6) produced experimental tumors in mice by applying Scottish shale oil to the skin. Attempts made to identify the specific chemical substances which could be responsible for skin cancers established fluorescence spectroscopy in the study of carcinogenic substances as an indispensable technique. Sir Ernest Kennaway (7) and his coworkers discovered the first cancer-producing hydrocarbon, 1:2:5:6-dibenzanthracene, and then isolated 3,4-benzpyrene from pitch. This discovery stimulated synthesis and testing of the hydrocarbons which were variations of dibenzanthracene. Benzene rings were added and subtracted, alkyl groups

were substituted at different positions in the nucleus of the molecule,
hexagon rings were replaced by pentagon rings, carbon atoms were replaced
by pentagon rings, and carbon atoms were replaced by nitrogen and sulfur.
According to Hieger (8), who reviewed in detail the history of these ef-
forts, the number of compounds which have been tested for carcinogenic
activity must be counted in thousands. Falk (9) has listed some of the
important carcinogens with their respective carcinogenic potency, and a
summary of this information appears in Table 1.

TABLE 1

CARCINOGENIC ACTIVITY OF POLYCYCLIC AROMATIC
HYDROCARBONS FOUND IN VEHICLE TIRE RUBBER (9)

COMPOUND	ACTIVITY
1,2-benzpyrene	very weak
3,4-benzpyrene	strong[a]
1,2-benzanthracene	weak
1,12-benzperylene	very weak
chrysene	weak
pyrene	negative
coronene	negative
anthanthrene	negative

[a]3,4-benzpyrene is the strongest as well as the most ubiquitous of
the carcinogens.

Though workers in modern society are not exposed to soot in the same
manner as were the chimney sweeps of a century ago, the problem is still
around in a more complex form. Carbon black is an ingredient of many

articles of civilization and can be a host for 3,4-benzpyrene and some

of the other carcinogens listed in Table 1. The manufacturing process,

properties, and nomenclature of carbon black are succinctly reviewed by

Nau (10) in reports of the results of his recent physiological studies

on skin contact absorption, injection, and ingestion of this still impor-

tant ingredient of commerce.

Carbon black is a component of two ubiquitous products of daily

consumption, the automobile tire and the food additive. Thompson (11)

has demonstrated the contribution of the automobile tire as an air

pollutant. Nau (12), in his studies of carbon black ingestion, points

out the extensive use of carbon black as a coloring agent for certain

foods (jelly beans, chocolate, candies, gum drops, licorice), and in

inks and in rubber goods used in food processing (tubing, conveyor

belts, jar lids).

Falk (9), in a study reporting that extracts of rubber stoppers are

carcinogenic for the skins of mice, also reports the isolation from rub-

ber stoppers and automobile tires of eight aromatic hydrocarbons. Py-

rene, 3,4-benzpyrene, chrysene and alkyl cyclopentenophenanthrene were

identified by ultraviolet absorption spectra and by melting point de-

terminations, and the identity of another crystalline product, 1,12-benz-

perylene, seems fairly certain. Alkyl pyrene, 1,2-benzpyrene, and alkyl

1,2-benzanthracene have not been positively identified because of their

oily nature. Since pyrene, 3,4-benzpyrene, 1,2-benzpyrene, and chrysene

have long been described in the literature as carcinogenic and since the

other four polycyclics are structurally related to carcinogenic compounds,

the carcinogenic properties of rubber stopper extracts are not surprising.

While the stoppers and the automobile tires used in the studies of Falk (9) contained processed rubber, both natural and synthetic unprocessed rubber has been studied by Bateman (13), Jarrijon (14), and Kaye (15), who report that the absorption spectra observed in their studies have not been those of any known carcinogen. It seems, therefore, that carcinogens are introduced during the processing of rubber, and, according to Barron (16) and Noble (17), might enter along with the catalysts, the pigments, the antioxidants, or the fillers.

Typical catalysts are mercaptobenzothiazole and tetraethylthiuramdisulfide, compounds containing sulfur and nitrogen, which are not at present suspect since their structure differs from that of any known carcinogen. Pigments used include both organic and inorganic compounds, some of the former being azodyes. However, the structure of these azodyes is not identical with that of any known carcinogen. Although some of the antioxidants are related to betanaphthylamine, a known carcinogen, Boyland (18) and Shear (19) have reported them to be inactive on tests for carcinogenecity.

The filler most widely used in the manufacture of vehicle tire rubber is carbon black, a product similar to soot in that some forms of it contain carcinogenic substances. While all carbon blacks are the product of oxygen deficient combustion of carbonaceous fuels, there are many varieties of the product and much variation in methods of manufacture. Nau (10) summarizes the four processes of manufacture as follows:

a) The impingement or channel process in which fine particle (highly agglomerated) blacks are made entirely from gas.

b) The lamp black process in which a relatively coarse (highly agglomerated) carbon is produced from a variety of oil products.

c) The furnace process, using either gas or oil or both, in which, by varying the operating conditions, blacks of graded sphere size can be produced.

d) The thermal process, in which hydrocarbon gases are used to produce coarse blacks which have relatively little tendency to agglomerate.

Because of the use of carbon black in rubber products the identification of polycyclic aromatic hydrocarbons (potential carcinogens) is of importance. Falk (20) analyzed 24 samples of commercial carbon black which included 7 channel blacks and 17 furnace blacks. He reported that no polycyclic aromatic hydrocarbons could be eluted from the channel blacks or from a few of the furnace blacks. In most of the furnace blacks, however, he identified 7 aromatic hydrocarbons: pyrene, fluoranthene, 3,4-benzpyrene, anthanthrene, 1,2-benzpyrene, 1,2-benzperylene, and coronene. This group of polycyclic aromatic hydrocarbons is the same as those which he also identified as present in processed rubber (stoppers and vehicle tires) except that coronene which he found in carbon black was not in rubber and chrysene and the phenanthrene derivative which he detected in processed rubber were not found in any carbon black.

CHAPTER III

THE CHEMISTRY OF POLYCYCLIC AROMATIC COMPOUNDS

The carcinogens called polycyclic aromatic hydrocarbons discussed in Chapter II are also sometimes referred to as polyaromatic, or polynuclear, hydrocarbons. The general structural formula for a molecule of a polycyclic aromatic hydrocarbon compound is as follows:

$$\left[\bigcirc\!\!-\!\!\bigcirc\right]_X \quad \text{or} \quad \left[\bigcirc\!\!\bigcirc\right]_X$$
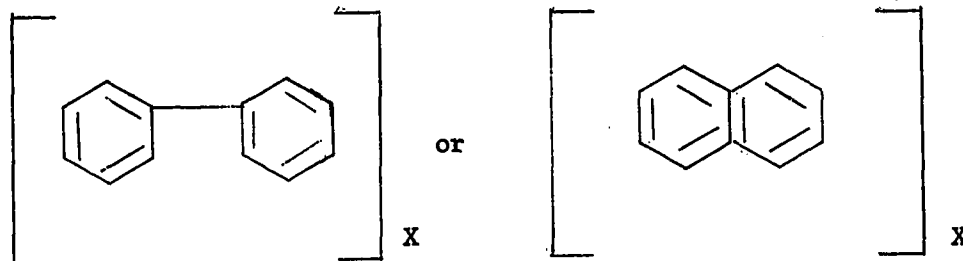
The polycyclic aromatic hydrocarbons, which are essentially odorless, are solids at room temperature, and although most of them are colorless, some are colorful. They have a low vapor pressure at room temperature, boil above 200°C and have high melting points. They are relatively insoluble in water and in the usual organic solvents but slightly more soluble in the aromatic hydrocarbons with lower molecular weight. An interesting property is their solubility in purines which decreases with the number of combined rings. Weil-Malherbe (24) reports that with an equal number of rings, the angular configuration is more soluble than

the linear arrangement. Their solubility in aqueous caffeine is used in the physical-chemical test for the carcinogenic potency of high boiling petroleum hydrocarbons. They absorb in the ultraviolet and infrared region of the electromagnetic spectrum and many possess the property of fluorescence. Some of them, as anthracene and diphenylhexadiene, possess the property of scintillation and form complexes (picrates, styphnates) which are used for isolation, purification, and quantitative analysis.

Colorimetric procedures consisting of identification of the hydrocarbon by means of nitration, treatment with piperonal, treatment with 3-nitro-4-dimethylbenzaldehyde, or isatin-fluorescence spectrometry, include both qualitative and quantitative methods. The sensitivity of some of the tests is in the range of parts per billion.

In a study of the chemical contaminants in city air, Wedgewood and Cooper (22) determined the polycyclic aromatic hydrocarbons spectrophotometrically after chromatographic separation on alumina. Bergmann and Gruenwald (23) described how a mixture of polycyclic aromatic hydrocarbons can be separated by paper chromatography and made visible by exposure to ultraviolet light.

Since the absorption spectrum of each compound is so individual that it is sometimes referred to as a fingerprint, infrared absorption is a particularly powerful tool for identification of polycyclic aromatic hydrocarbons. It is a difficult technique to apply, however, because the compounds are not very concentrated in the source material and they must be recovered in a highly refined condition. A paradox then arises in the analytical work since no identifying spectrum can be obtained unless the substance is "pure" and no purifying technique can be perfected

unless the analyst knows the "identity." This same problem may arise in gas chromatography.

Eby (24) used several varied polarographic procedures for predicting tumor potency of high boiling petroleum fractions containing polycyclic aromatic hydrocarbons. These methods made use of the differing reduction potentials of anthracene and other aromatic hydrocarbons. Polarography appears to be about as suitable for prediction of tumor potency as the Esso "caffeine number" method reported by Eby.

Sawicki (25), in a recent paper, described very sensitive color tests for the qualitative and quantitative determination of polycyclic aromatic hydrocarbons. Brilliantly colored diarylmethane dyes are formed by the reaction of the hydrocarbon with benzal or piperonal chloride in trifluoroacetic acid. Colored complexes are formed by the interaction of polycyclic aromatic hydrocarbons with 2,4,7-trinitrofluorenone, according to Gordon and Huraux (26). Some of the complexes also show brilliant ultraviolet fluorescence. Qualitative information about the structure of the unknown compounds can be derived by observing the color and fluorescence of the trinitrofluorenone complex on a paper chromatogram and by determining the solubility of the complex in isooctane and ethyl alcohol.

Bergmann and Gruenwald (23) gave the details of a refinement of paper chromatography in which a mixture of polynuclear hydrocarbons was separated and the individual spots made visible with a spray of a 20 per cent solution of antimony pentachloride in carbon tetrachloride.

In some analytical studies, large quantities of the polycyclic aromatic hydrocarbon compounds are needed and large amounts of material

must be treated to obtain adequate material for study, particularly in initial studies on compounds whose identity is not known and where several milligrams of material are needed for proving the structure, determining molecular weight, and establishing melting point and boiling point data. Cleary (27), whose method and results are typical of column work in this area, used a long column, 26.5 inches by 0.15 inches, filled to a depth of 23 inches with a slurry of activated aluminum oxide in cyclohexane. The aluminum oxide, prior to being packed in the column, was heated in an air oven at 120°C for 72 to 96 hours and then brought to equilibrium over 50 per cent sulfuric acid for at least 36 hours. The moisture content of the alumina was then 13 to 13.5 per cent. Care was taken to keep the column from becoming dry. The sample was dissolved in cyclohexane and passed into the column in this solvent. The column was then developed by a mixture of cyclohexane and ether. A 3660-Angstrom ultraviolet lamp was used to locate fluorescent zones on the column and to follow the passage of the eluted material. Twenty-five successive fractions were then collected from the column and examined spectrophotometrically in the ultraviolet regions. The substances were identified by the position of distinctive absorption bands.

Most column procedures are of the types described above. However, Kotin (28), Cooper (29), Wedgewood and Cooper (30), Lindsey, et al. (31), and Kotin, et al. (32) (33) (34) pioneered studies in which column chromatography on activated alumina or silica jel, with a great variety of developers ranging from benzene and isooctane mixtures through those involving ether, methanol and petroleum ether, was used for primary division. The columns used in these studies were short columns, and in many

cases poor separation was the rule rather than the exception.

The failure of the short columns to achieve total separation of the compounds represented among the polycyclic aromatics led to the development of methods using paper chromatography. Considerable success in the use of these methods is reported by Tarbell (35) in his paper on the separation and purification of 3,4-benzpyrene and other polycyclics. In this work a strip of Whatman's No. 1 filter paper was saturated with redistilled N,N-dimethylformamide and allowed to air dry for one hour. One microliter of sample in benzene was applied at a marked spot near the lower end of the paper and dried by a stream of nitrogen. The paper was then left overnight for equilibration in a chamber saturated with the developer of hexane saturated with N,N-dimethylformamide, after which the bottom end of the paper was immersed in the developer. When the solvent front had ascended about 16 inches on the paper, the strip was removed and inspected under ultraviolet light which revealed the polycyclic compounds readily visible as separate spots.

There are many other methods of paper chromatography. Some employ descending solvent fronts while others vary the proportions of solvent constituents. The chief disadvantages of techniques involving paper chromatography are the long periods of time required for the development, the instability of the paper to reagents, and the bulky and cumbersome developing tanks.

Major disadvantages inherent in paper chromatography can be overcome by the newer thin-layer methods, in which the theory of separation is essentially the same as that for column and paper chromatography. In the thin-layer technique the adsorbent, either cellulose, closely

paralleling separations on paper, or the alumina or silica usually re-
stricted to columns, may be spread in a thin layer on a glass plate and
easily treated with reagents, a procedure destructive to paper strips
and restricted or impossible on columns. The cumbersome apparatus of
columns and jars may be replaced by the smaller, more convenient and
more economical rectangular tanks. Further advantages lie in the ease
with which experimental adsorbents and developers may be tried out on a
pilot basis with small glass plates such as microscope slides. However,
thin-layer chromatography will not solve all the problems inherent in
the study of polycyclic aromatic compounds. Some problems remain sol-
vable by columns only, particularly those in which larger quantities on
the order of milligrams are needed.

In the use of thin-layer chromatographic techniques, Sawicki (36)
has developed a successful method for the determination of 3,4-benzpyrene
in air pollutants and has also reported considerable success with a num-
ber of other polycyclic aromatic hydrocarbons. His method was essentially
one of benzene extraction of the collected particles, followed by spotting
of the benzene extract on cellulose plates and developing with a mixture
of N,N-dimethylformamide and water. He also used, from among the many
possible variations, such combinations of adsorbents and developers as
alumina with pentane and ether as developer and cellulose acetate with
ethanol, toluene, and water as developer.

In some cases polycyclic aromatic compounds resist separation from
the material in which they are embedded, particularly those present in
processed rubber. At the Oklahoma University Institute of Environmental
Health, dimethyl sulfoxide and N,N-dimethylformamide are being explored

as possible solvents for the direct extraction of polycyclic compounds
from carbon black and processed rubber. N,N-dimethylformamide has also
been found useful as a solvent when determining the absorption spectra
of recovered polycyclic compounds.

The most sophisticated technique for the determination of poly-
cyclic compounds involves the use of gas-liquid chromatography, a type
of instrumentation which combines the principles of both separation and
identification. In a gas-liquid partition system, the separation of the
components is achieved on the basis of the differing partition coeffi-
cients between a stationary phase and a mobile phase in the column. The
stationary phase is a liquid normally having a very high boiling point.
The mobile phase is an inert gas generally referred to as the carrier.

Gas-liquid partition columns are of two general types, the packed
column and the capillary column. In the packed column, the stationary
phase is uniformly coated upon an inert granular solid support most
commonly prepared from a material such as diatomaceous earth with par-
ticle size ranging from 20 to 140 mesh or, in some cases, glass beads
or Teflon. Diatomaceous earth is advantageous in that it will retain
up to 30 per cent (by weight) of liquids and still be dry enough for
convenience in packing into a tube. In capillary column types, the
liquid phase is coated directly onto the walls of an open tubular or
capillary unit with no solid support provided.

The column is installed in the instrument in such a way that the
carrier gas continuously flows through it. A small quantity of the
sample mixture to be separated is injected into the carrier gas
stream through the injection port of the instrument from which it

is carried in a body onto the column. The individual components of the sample move through the column at different velocities, the velocity of the component dependent upon its partition coefficient (symbolized as K) between the liquid phase and the carrier gas. The partition coefficient of a compound is defined as the ratio of the concentration of molecules in the liquid phase to the concentration in the gas phase at any given moment. The larger the partition coefficient, the more slowly the component will traverse the column. Individual components emerge from the column and pass through the detector which, in conjunction with a recorder, generates a hill and valley chart on which the hills or peaks represent pure compounds.

The chief problem in adapting the gas chromatograph to a particular situation is the selection of a suitable column. It has been reported that a silicone rubber column manufactured by Varian Aerograph of Walnut Creek, California, when used with a flame ionization detector, is suitable for separation of polycyclic compounds including 3,4-benzpyrene (37). Specifications for this column are 3 per cent SE-52 on Aeorpak 30, 100-120 mesh packed in a stainless steel column 10 feet long and 0.0625 inch in outside diameter.

A search of the literature reveals that no previous efforts have been made to determine the ultimate fate of polycyclic compounds after their passage into rubber. That rubber deteriorates is well known. What happens to the polycyclic aromatic hydrocarbons during this process is of potential public health interest.

CHAPTER IV

PURPOSE AND SCOPE

This study was conceived as one aspect of research in progress at
the University of Oklahoma Institute of Environmental Health in which
some of the health problems related to rubber dust exposure are being
studied. The broad study, begun in January 1962, is entitled "Occupa-
tional Health Hazards of Rubber Dust." One of its objectives is to
determine whether rubber dust abraded from commercial processed rubber
and deposited by inhalation in significant amounts in the lungs of
various species of animals can be affected by the subsequent inhala-
tion of exhaust gases. One way this effect could be manifest would
be by the elution of polycyclic aromatic hydrocarbons and a consequent
increase in tumor formation. Further, it seeks to determine whether
these dusts are significant occupational health hazards as air pollu-
tants and carcinogens.

The presence of polycyclic aromatic hydrocarbon compounds on car-
bon black and subsequently in processed rubber makes abraded rubber a
possible source of carcinogens. The objectives of this particular in-
vestigation were, therefore, as follows:

a) To develop a procedure for readily determining, with economy

of time and equipment, whether polycyclics are present in

questioned materials, particularly carbon black and processed

rubber.

b) To identify the polycyclic compounds found.

c) To determine whether the assortment of polycyclic compounds found in carbon black is the same as the one present in the processed rubber in which the carbon black was employed as a filler.

d) To establish the influence of the processes of manufacture, age, and abrasion on the polycyclic compounds as they pass by way of carbon black through vehicular tire rubber.

A review of the literature has indicated that polycyclic aromatic compounds tend to occur together as a group or assortment, particularly during the incomplete combustion of carbonaceous materials. We might therefore expect to recover an assortment or "set" of polycyclic aromatic compounds from a sample of carbon black or vehicular tire rubber in which carbon black was used. A means of comparing the assortments of poly-cyclics would provide some information about the influence of processing, aging and wear on these compounds. Before inquiry can be made, a technique must be developed for determining the polycyclic "assortment," "population," or "set." Older studies have done this by identifying each compound individually. This study will deal with methods for comparing mixtures of polycyclics from two sources as well as for identifying compounds wherever possible.

Completion of these objectives will make available a method for readily monitoring the assortment of polycyclics present in various circumstances. Lijinsky (38) reported the recovery of 3,4-benzpyrene and other polynuclear hydrocarbons from charcoal broiled meat. He

raised the question of the origin of the polycyclics and presented some data for the view that the polycyclics were formed from the pyrolysis of fats in the heat. A technique for reviewing the polycyclics assortment in the charcoal before its use as a fuel would answer some of the questions as to when they originated.

Niels Dungal (39) presented data indicating that the polycyclics found in the rain water used by cancer victims were formed in some manner other than by the pyrolysis of fat. He showed that one large geographically isolated group suffered a high incidence of gastric cancer because of their monotonous diet of smoked meats, particularly mutton and salmon. The cancers were presumed attributable to the presence of an irrita..._ anthracene (a polycyclic and mild carcinogen) and 3,4-benzpyrene (a strong carcinogen) in smoked meats. The origin of the 3,4-benzpyrene was not immediately apparent. However, another geographic group in Iceland which ate little smoked meat had an inexplicably high incidence of gastric cancer. Their source of 3,4-benzpyrene and other polycyclics was drinking water obtained from the roofs of their houses which were heated with fuel oil, the soot from which found its way to the rain barrel and ultimately to the gastric mucosa. Researches of this kind presage a wide use for analytical procedures which can be quickly and conveniently applied. The scope of the distribution of polycyclic compounds is such that rapid survey methods are needed for defining their whereabouts, concentrations, and eventual degradation.

The increased use of asphalt and other petroleum products is such that ever increasing numbers of people are exposed occupationally or routinely to the substances themselves or to their by-products, thus

increasing the need of a technique for comparison of polycyclic compounds in these substances. An examination of criminal records reveals that even in forensic areas it would be helpful to have such a technique for comparing the roofing compounds of burglarized buildings with the besmirched clothing of robbery suspects.

Falk (40), as a result of a study of aqueous protein solutions, presents some evidence that 3,4-benzpyrene was rapidly eluted from rubber dust under circumstances somewhat similar to those encountered in the mammalian lung. A method for rapid comparison of polycyclic sets from different sources would be exceedingly useful in studies of this type.

CHAPTER V

MATERIALS AND PROCEDURES

Since the chief purpose of this study was to compare assortments of polycyclics, it was necessary to select three fundamental starting materials: carbon black, abraded rubber from new tires, and abraded rubber from old tires. The carbon black selected was furnished by the B. F. Goodrich Company of Miami, Oklahoma, who identified it as of the type and grade used in their vehicular tires. The Goodrich Company further specified that the carbon black was an Intermediate Super Abrasion Furnace black manufactured by the furnace process. Falk (9) has shown that furnace blacks are a likely source of polycyclic aromatic hydrocarbons.

One sample of the rubber abradings used for the analytical work was furnished by the United States Bureau of Standards who identified it as having been abraded from new tires manufactured by Goodrich, Firestone, Goodyear, and U. S. Royal in such manner as to duplicate the formation of abradings by wear on highways. It was, however, mixed with abrasive materials which served in the mechanical function of abrading.

Another sample of abraded rubber was provided by local retread shops and consisted of an accumulation of abradings from a wide variety of old tires. This sample was considered representative of tire rubber which possessed the properties of both age and wear.

In further expansion of this work, it was decided to apply the

analytical method developed for recovering and displaying polycyclic aro-
matic compounds to biological tissue in an effort to monitor the ultimate
fate of the compounds in a functioning organism. Tissue from the lungs
of monkeys exposed to both channel and furnace black was available for
study.

Treatment of all samples was the same in the initial extraction to
recover the polycyclics. A weighed sample was placed in the thimble of
a Soxhlet extractor and extracted for a 24-hour period with boiling re-
distilled benzene.

It was found from base line calculations that all of the benzene
extracts in this study possibly contained polycyclic aromatic compounds.
This was preliminary evidence that the polycyclic content of both the
carbon black and vehicular rubber was the same. The next objective was
to apply thin-layer chromatography in initiating separation of the poly-
cyclic compounds into an assortment of individual substances. The fact
that each polycyclic compound exhibits a distinct fluorescence spectrum
is of great value in following the compounds through analytical treatment.
Several adsorbents and developing solutions were tried for the thin-layer
chromatographic separation before a suitable one was found. Since the
outcome of these several trials may be useful in applying thin-layer
methods to other materials, the details are reported in the section on
results. It was found that the best thin-layer adsorbent for these par-
ticular studies was a powdered cellulose of the tradename MN Cellulose 300
manufactured by the Machery Nagel Company and distributed in the United
States by the Brinkman Instruments Company of Westbury, New York.

The adsorbent slurry of cellulose was spread on glass plates which

were about 0.25 inch in thickness and 8 inches square. The adsorbent was applied to the glass plates by use of an adjustable spreader manufactured by Desaga and distributed by Brinkman Instruments Company. The best results were obtained with an adsorbent thickness of 250 microns, a setting readily available on the adjustable delivery gate of the spreader. The cellulose slurry was prepared by mixing 15 grams of cellulose powder and 90 milliliters of water in a Waring Blendor. This quantity of slurry was enough for 5 plates 8 inches by 8 inches in dimension. After the plates were coated with the slurry, they were oven dried at 110°C for 30 minutes and then placed in a desicator until ready for use.

Benzene extracts of carbon black and other samples were spotted directly on the cellulose plate in 5-microliter quantities by the use of a micropipet operated by a screw type control. Because of the awkwardness and lack of precision inherent in the recommended operation of the micropipet and its control as a unit, the procedure was modified. A short section of small diameter rubber tubing was connected to the pipet at one end and to the bottom of the pipet control at the other as shown in Figure 1. Movement of the screw pulled the plunger for a vacuum which was then transferred to the pipet by way of the rubber tubing. The pipet control was mounted permanently on a ring stand with the length of rubber tubing serving as a quick and convenient attachment to the pipet. The unit was very maneuverable with the screw control unit mounted in a fixed position and operable with the left hand. The tip of the pipet was moved by the right hand to the desired position on the cellulose plates and in a swift motion could be removed from the tubing for rinsing or replacement. As a measured quantity of solution was delivered to the cellulose
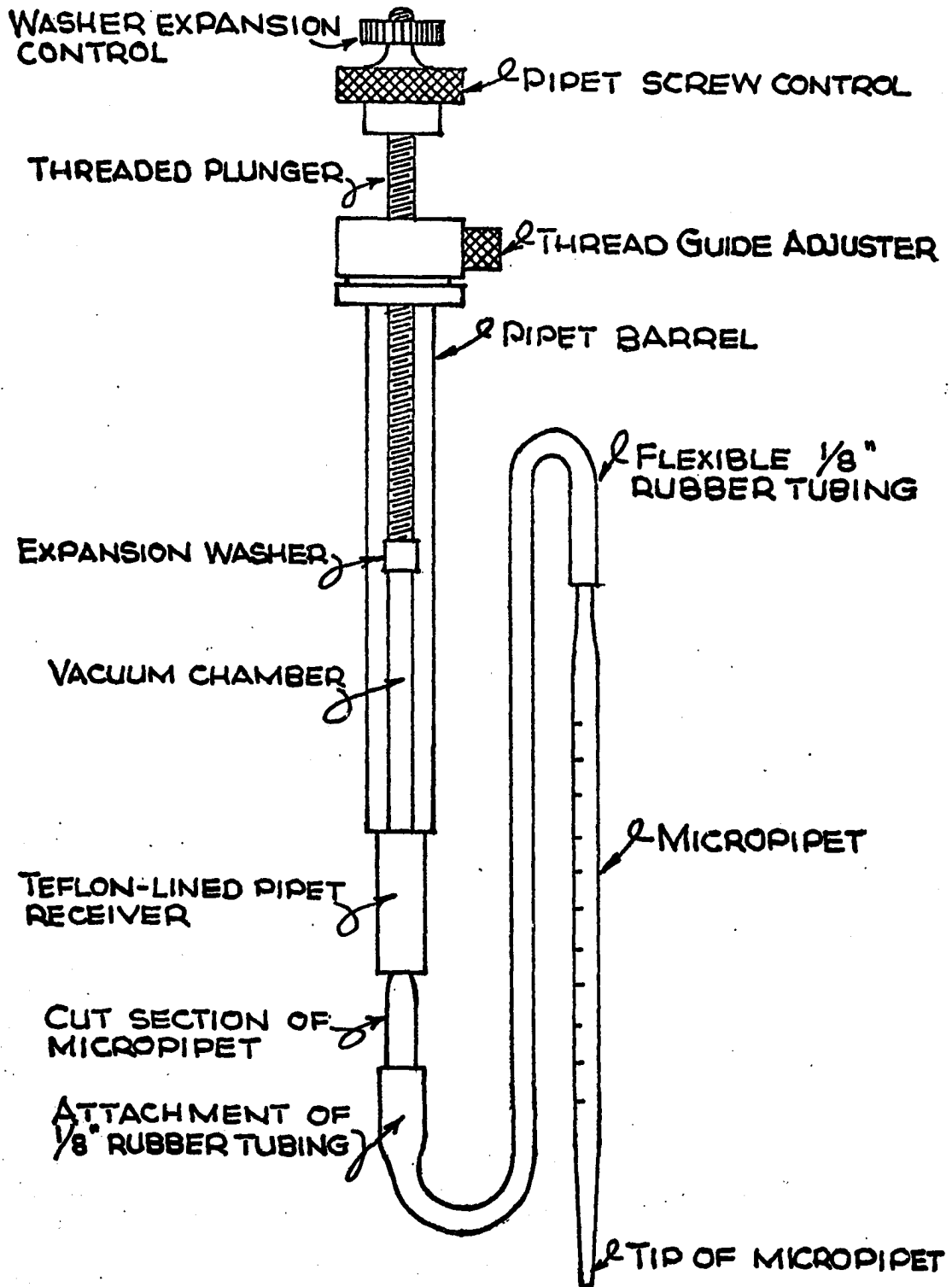
Figure 1. Pipet control with an assembly of tubing and a micro-pipet.

plate, it was evaporated by a current of warm air from a hair dryer. The

plate was then ready for development in a chromatographic chamber.

The chambers for this study were prepared from hollow glass building

blocks, 10 by 12 by 4 inches in outside dimension and large enough inside

to hold the glass plates on which the adsorbent was spread. One end of

each block was cut off and replaced with a plate glass lid. The chambers

were lined with filter paper which was saturated with the developer, a

mixture of N,N-dimethylformamide and water in equal portions. The bottom

of each tank was covered with the developer to a depth of 0.125 inch. .

A piece of Saran wrap was stretched over the moistened rim of the tank

and weighted with a glass lid so that the atmosphere in the tank might ..

become saturated with the developer. In approximately 2 hours, or as

soon as an equilibrium was established, two of the cellulose plates,

their lower margins spotted with the samples, were placed in each tank

for development. They stood in the developer to a level just below the

spots, and the developer moved up the adsorbent, traveling to within 1

inch of the top in 2 to 3 hours. At the end of this time they were ex-

amined in the dark room under the rays of a 3660-Angstrom ultraviolet

lamp. Examination revealed a series of fluorescent spots or zones de-

noting the presence of an assortment of fluorescent compounds.

Basically chromatography consists of a two-phase system. One phase

is fixed and is termed the stationary phase. This phase may be a solid

as in adsorption chromatography (Tswett's original method) or a liquid

held by a solid as in partition chromatography which may be contained in

a column or formed in sheets or strips of cellulose as in paper and thin-

layer chromatography. Polycyclic compounds separate on the thin-layer

plate because of the differing partition coefficients between the mobile

phase and the water saturated cellulose. The mobile phase function was

performed in this study by the N,N-dimethylformamide and the stationary

phase by the water saturated cellulose.

When viewed under ultraviolet light, the spots developed in this

study showed a separation into six distinct components or zones as

shown in Figure 2. Each component was visible under ultraviolet light

as a separate and distinct fluorescent spot. There was considerable

variation in color among the six spots or zones observed.

As a standard for comparison a series of known polycyclic compounds

was spotted and developed on thin-layer plates, each standard being com-

pared with a parallel development from the benzene extract of the materi-

als studied. Polycyclic compounds used for standards were as follows:

fluoranthene, 3,4-benzpyrene, pyrene, benz [a] anthracene-7,12-dione,

1,2-benzpyrene, anthanthrene, coronene, anthracene, naphthalene, phenan-

threne, and 1,12-benzperylene. These compounds were selected because

Falk (9) (20) had reported their presence in rubber and in carbon black.

Eastman Kodak Company of Rochester, New York, and the Aldrich Chemical

Company of Milwaukee, Wisconsin, furnished the standard compounds which

were specified as zone refined and highly purified.

As soon as the separated spots or zones had been numbered for posi-

tion and compared with the standards, they were delineated with a dis-

secting needle by clearing off a narrow path surrounding the zone.

The delineated zones were removed with a microvacuum cleaner ap-

paratus similar in design to that described by Hagen, Dunlap and

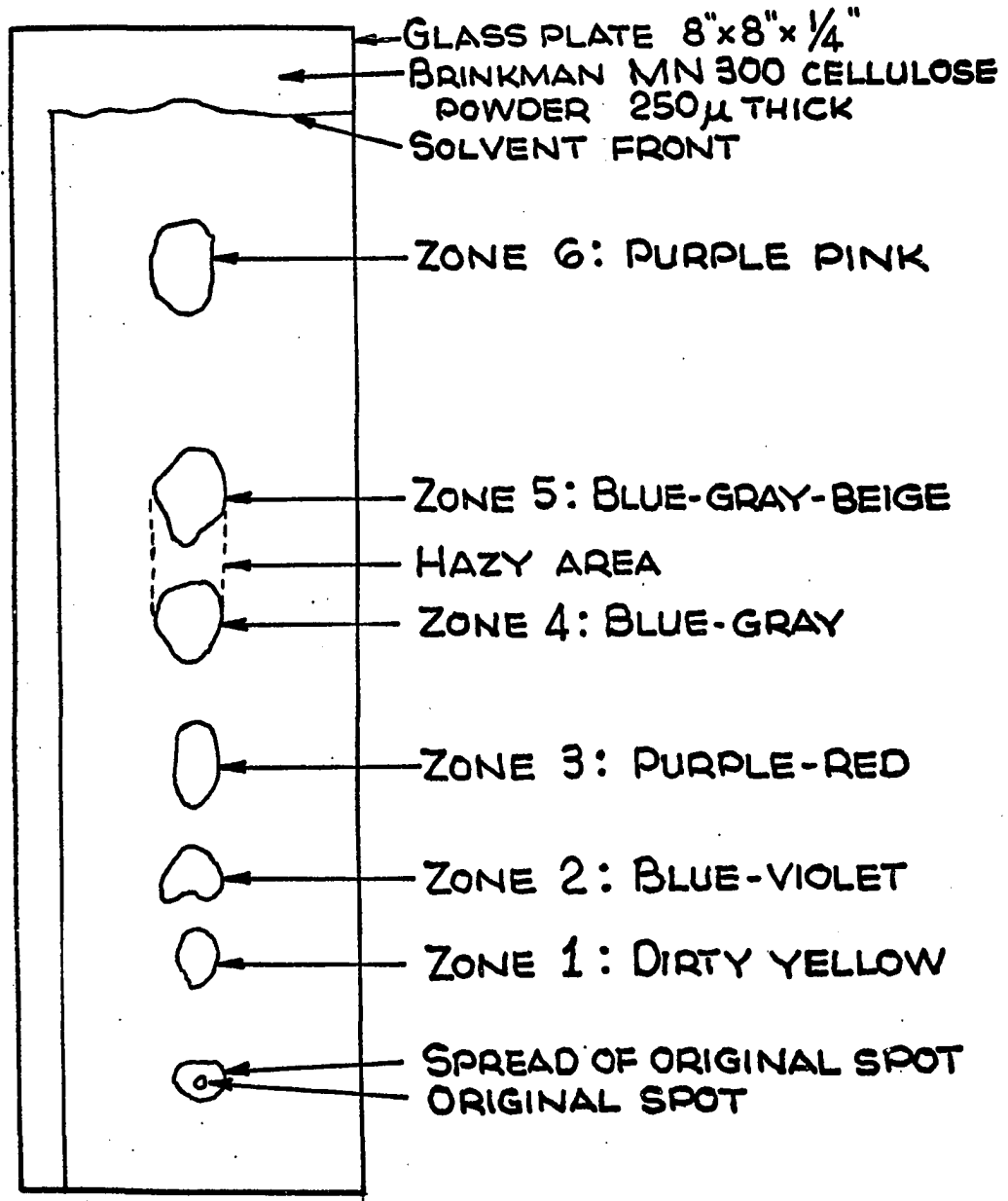Wender (41) and depicted in Figure 3. A small diameter circle of

Figure 2. Schematic diagram of zones and their colors as separated on the thin layer chromatograph plate.

INTAKE ORIFICE, 1.5 MM BORE CUT TO 45° ANGLE

SPRING HOOK

$ 7/15 GROUND JOINTS

11.5 CM.

GLASS FIBER PLUG

TO ASPIRATOR

WASH SOLUTION ADDED VIA SYRINGE

MICROVACUUM CLEANER

NO. 8 RUBBER STOPPER

3 MM O.D. POLYETHYLENE TUBE

TO ASPIRATOR

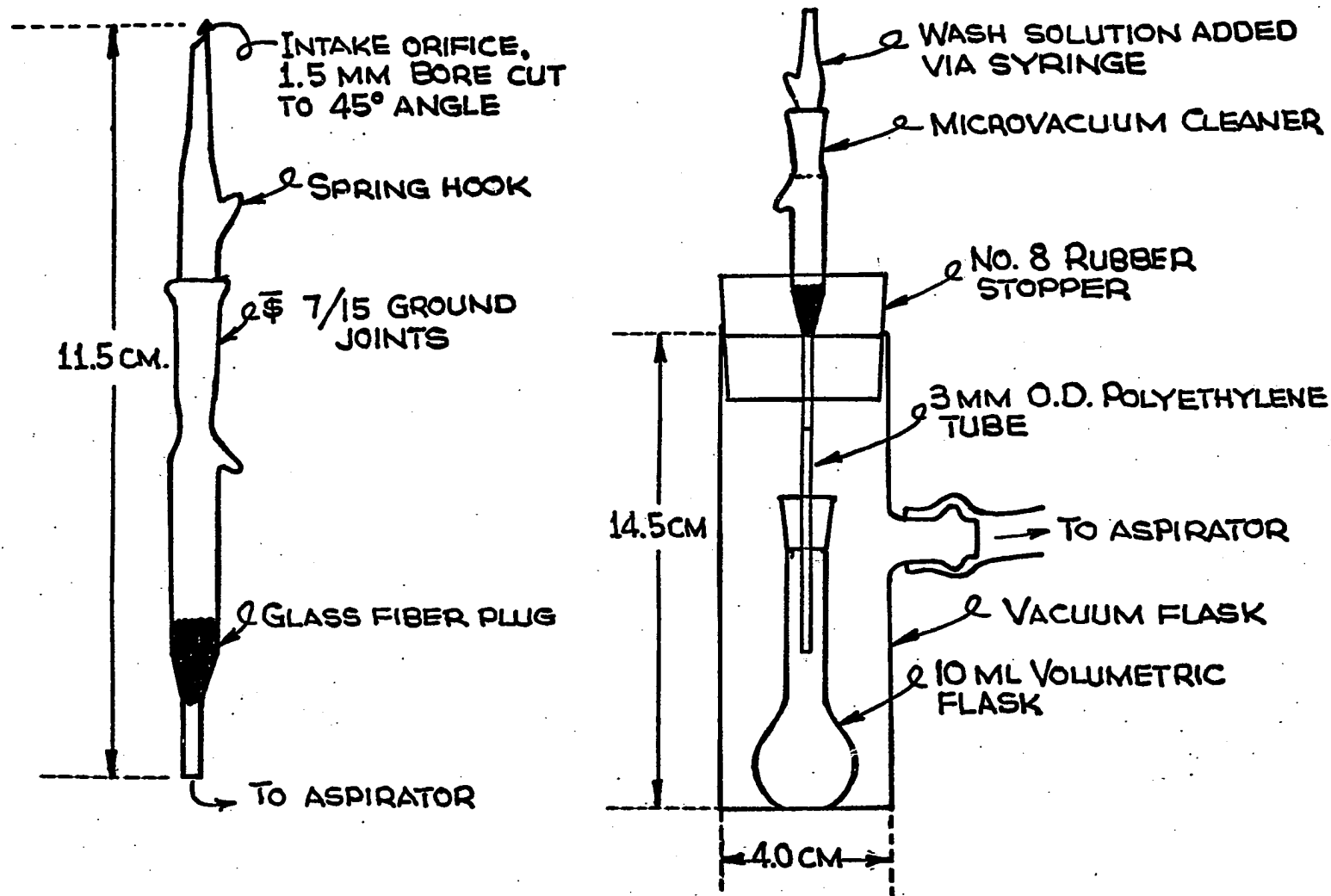14.5 CM

VACUUM FLASK

10 ML VOLUMETRIC FLASK

4.0 CM

Figure 3. The vacuum cleaner unit for the removal of adsorbent and its assembly into the elution chamber.

glass fiber (cut from a Cambridge filter, Phipps and Bird, Incorporated, Richmond, Virginia) was pushed into one end of the vacuum cleaner unit until a firm fit was established.

A vacuum line was attached to the harvesting unit and the polycyclic-containing adsorbent was vacuumed from the delineated zones to accumulate in the filter trap. The vacuum line was then removed and the filter-end was inserted through a vacuum chamber cork into a 10-milliliter volumetric flask as shown in Figure 3. When a tight seal was established in the rubber stopper, the vacuum hose was attached to the side arm of the vacuum bottle. Hot methanol was then added to the distil end of the vacuum cleaner unit and drawn by a careful application of the vacuum onto the adsorbent and the filter. The vacuum was shut off for a brief period to allow the compound in the adsorbent to go into solution. As soon as solution was accomplished, the vacuum was turned on again and the solution was drawn into the volumetric flask. For convenience a complete set of vacuum apparatus (shown schematically in Figure 4) was available for each zone numbered on the plates, and each zone was collected in its own corresponding volumetric flask of solvent. The collected adsorbent was washed with hot methanol until the washings were no longer fluorescent under 3660-Angstrom ultraviolet light. The volumetric receiving flask was then made up to final volume with methanol.

Study of the compounds thus obtained was continued by ultraviolet spectrophotometry. The ultraviolet absorption spectrum in the range from 340 millimicrons to 240 millimicrons was obtained by placing some of the sample in methanol in a 3-milliliter Cuvette and scanning in a Model DK2 Beckman (double beam) spectrophotometer.
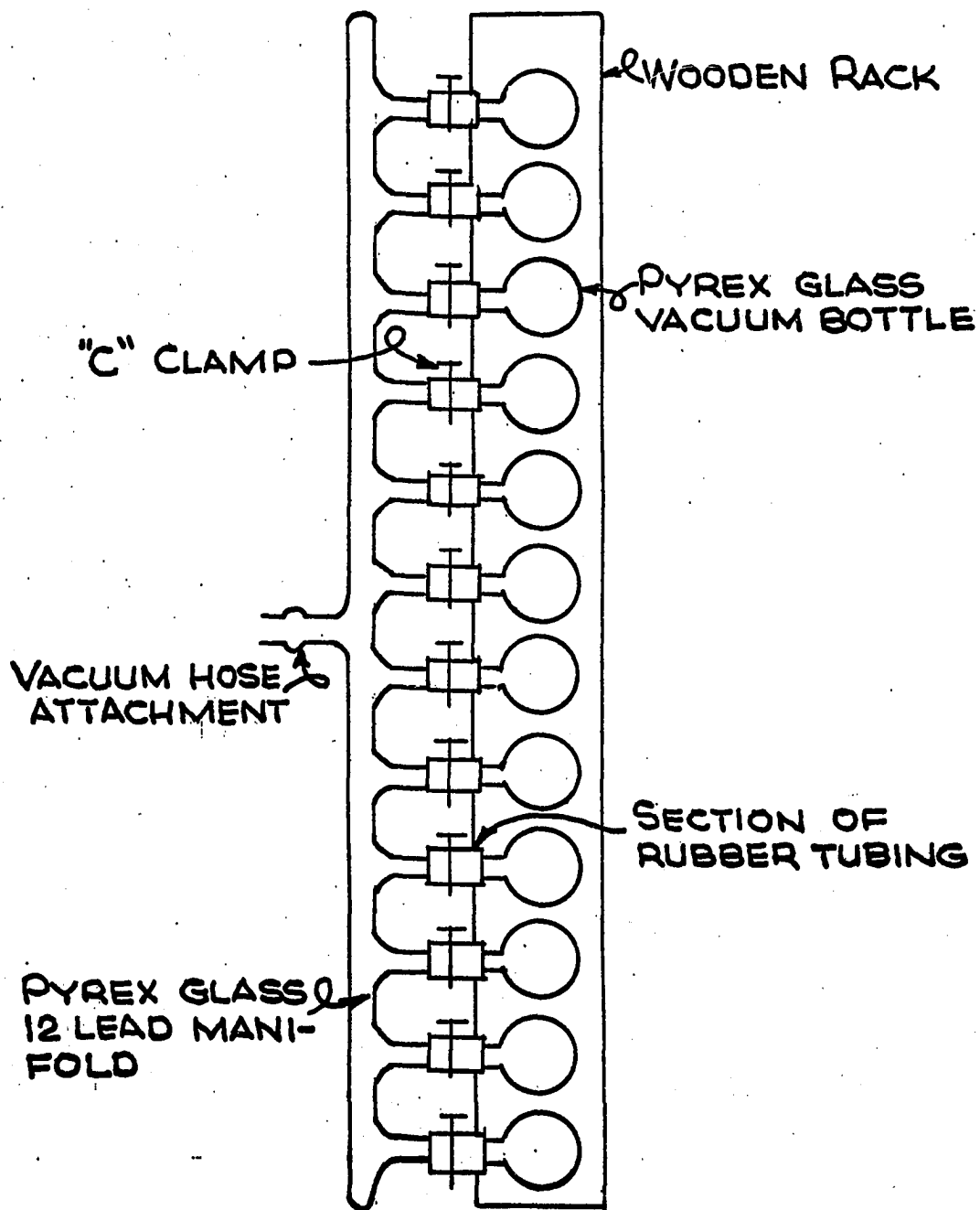
Figure 4. Vacuum chamber and manifold assembly for the elution
of recovered products into volumetric flasks.

Early in the study it was realized that the zones emerging on the
thin-layer plates did not in every case represent pure compounds. In
order to obtain more refined substances it would have been necessary to
develop a combination of other adsorbents and developers for further thin-
layer applications. The possibility of other adsorbents such as aluminum
oxide and silica and other developers such as pentane, alcohol, and ether
was to some extent explored and will be discussed later.

In view of the purpose of the study, which was to compare the
assortment of polycyclics in carbon black, in new rubber, and in rubber
which was to some extent aged and abraded, it was decided to approach
the comparison through the behavior of the compounds on the thin-layer
plates and by means of data on their respective absorption spectra.
Comparison of the standards with the unknown zones from the material
studied showed that there were some compounds present in the materials
from carbon black and rubber which were not represented among the stand-
ards available. Though the exact nature of these compounds was not known,
other than through their fluorescent properties and possible absorption
spectra, it was nevertheless possible to compare the two respective groups
of compounds by noting their respective positions, colors, gradations, ab-
sorption spectra, and concentrations.

In several instances it was possible to identify polycyclic com-
pounds which were common to all three of the materials extracted by com-
parison of the thin-layer chromatograms of the recovered products with
those of known polycyclic compounds. The distances and colors of zone
migrations were particularly useful features. The tentative identifica-
tions were verified by comparison of the absorption spectra of the

recovered products with that of known polycyclic compounds. In this manner it was also possible to compare absorption spectra of the recovered products even though no standards were available.

According to Leigh-Dugmore (42) a typical formulation for vehicular tire rubber is 35 parts of carbon black to a total of 151.70 parts by weight, or approximately 23 per cent. The weights of carbon black, rubber, and lung tissue extracted are shown in Table 2.

The monkey lungs shown in Table 2 were autopsy specimens from animals exposed to channel black and furnace black respectively. The lung tissue of these specimens was processed in a manner different from that used with the other samples. The lung tissue was not treated in a Soxhlet extractor with benzene but was first digested with alcoholic potassium hydroxide according to the method of Blackstock (43), and a determination of the weight of carbon black made. This fraction of carbon black was then extracted with hot benzene and the volume of extract reduced to 50 milliliters as were all of the other extracts. The lungs were included in the study primarily to investigate the applicability of the thin-layer methods to biological problems of carbon black exposure. The results, therefore, of the biological specimens are not of the same implication as those arising from the comparison of carbon black and rubber. Attempts were made by means of N,N-dimethylformamide extractions to improve the recovery of polycyclics from lung tissue, but this was not very successful because of the formation of emulsions. By observing thin-layer chromatograms of the carbon black extracts of monkey lungs it was expected that the presence or absence of some polycyclic compounds could be observed.

TABLE 2

WEIGHTS OF MATERIALS EXTRACTED

MATERIAL EXTRACTED	WEIGHT OF MATERIAL EXTRACTED (Grams)	WEIGHT OF CARBON BLACK PRESENT (Grams)
Carbon black (furnace) furnished by Goodrich Tire Company	329.5	329.5
New tire rubber abraded by the Bureau of Standards	297.0	68.5
Rubber abraded from old tires	316.7	73.1
Monkey lungs (furnace black)	64.4	5.4
Monkey lungs (channel black)	68.3	5.7

Certain assumptions were apparent in this exploratory work. There is considerable variation in the amounts of furnace black employed in the manufacture of automobile tires. There is some variation in the polycyclic content of furnace black prepared even from day to day. It is assumed that the carbon black furnished the laboratory by the B. F. Goodrich Company is of the same general type as that used in the tire rubber with which the comparison was made. It is anticipated that this preliminary work will provide a basis for the idealized experiment, i.e., one in which all of the carbon black studied will be factually known to be the same as that used in the tires.

It is to be noted also that the abraded rubber furnished by the Bureau of Standards had mixed with it an abrasive powder, alumina, which was used in the accelerated laboratory wearing process. For that reason

the calculation of the amount of carbon black in that particular sample

is only a first approximation. The presence of alumina also reduces the

efficiency with which the polycyclic compounds can be recovered by ben-

zene and N,N-dimethylformamide extraction. Yields of polycyclics in this

study from rubber containing abrasives were minimal.

Early in the study attempts were made to proceed in the analysis

directly from the benzene extract to the thin-layer plate. These attempts

were successful in the case of carbon black extract, but they failed com-

pletely in the case of benzene extracts of rubber products. No separation

of the polycyclics into zones could be observed when the extract was

spotted directly onto cellulose. The polycyclics were found in this par-

ticular instance to be present in a long, blue fluorescent, homogeneous

smear on the plate. Attempts to rectify this by first passing the benzene

extract of rubber serially through columns of alumina and silica were un-

successful. However, it was possible to prepare the benzene extract of

rubber for separation on thin-layer plates by utilizing solvent systems.

Both dimethyl sulfoxide and N,N-dimethylformamide were explored for this

purpose and N,N-dimethylformamide proved more suitable. The procedure is

described below.

The benzene extract of rubber was carefully evaporated and the

residue taken up in about 50 milliliters of warm isooctane. As soon as

all of the residue was in solution the volume of isooctane was reduced

with gentle heat to 15 milliliters. This portion was then extracted in

a 125 milliliter separatory funnel three successive times with 15 milli-

liters of a solution of 80 per cent N,N-dimethylformamide in water. These

three successive extractions were added to a 250-milliliter separatory

funnel. To this accumulation were added 50 milliliters of isooctane and 50 milliliters of water in that order, and the funnel was shaken vigorously for 1 minute. The phases were then allowed to separate and the aqueous portion was discarded. By this procedure the polycyclic compounds were back extracted into isooctane which was then dried with anhydrous sodium sulfate. This volume (50 milliliters) of isooctane was then reduced to 15 milliliters and the extraction with N,N-dimethylformamide was repeated two additional times. At the conclusion of these extractions the original benzene had been replaced by isooctane, and the final volume of isooctane was adjusted to 50 milliliters which was the volume of the original benzene extract. The isooctane was then spotted on thin-layer plates, and the polycyclics were found to separate into the six zones or constituents which were observed to be characteristic of the carbon black extracts.

CHAPTER VI

RESULTS AND DISCUSSION

Some of the adsorbents and developers explored for possible use in this study are shown in Table 3. Three principal adsorbent groups, cellulose, aluminum oxide and silica, are represented here. The most satisfactory adsorbent for the thin-layer separation of extracts of carbon black, rubber, and biological tissue was found to be the cellulose powder designated by Brinkman Distributors as MN Cellulose Powder 300. Many adsorbents have a binder, usually calcium sulfate, mixed with the cellulose to give the product the property of adhering to the glass plates. The MN 300 cellulose of Brinkman, however, is prepared without a binder and adheres to the plates sufficiently well if the plates are thoroughly clean. There was some tendency for the adsorbent layer to flake off, but this was overcome by careful handling. The microcrystalline cellulose of FMC Corporation, Marcos Hook, Pennsylvania, is also a product without a binder, and it has the advantageous property of forming a closely adhering parchment-like surface. It has the unsatisfactory property of not separating polycyclic compounds, at least under the conditions encountered in this study.

Sawicki (25) has reported that acetylated cellulose adsorbents were very satisfactory for the thin-layer separation of polycyclics from air pollution products. A series of cellulose adsorbents ranging from 20

to 40 per cent acetylated was tried on carbon black and rubber extracts but with very little success.

Sawicki (25) has reported that a mixture of N,N-dimethylformamide and water was suitable as a developer for separation of polycyclics recovered from air pollutants. It was therefore logical to investigate this solvent combination for the thin-layer separation of the products recovered from carbon black and rubber. Several proportions were studied for their resolving properties on different adsorbents, and the most satisfactory developer was found to be a mixture of equal proportions of N,N-dimethylformamide and water when used on the adsorbent MN Cellulose 300 by Brinkman. The results are shown in Table 3.

So little success was experienced with the use of aluminum oxide as an adsorbent that it was concluded that the polycyclics were not stable under the conditions of development.

The Silica Gel G of Brinkman gave no satisfactory results. Both the alumina and silica adsorbents were used in combination with different developers, all of which failed to give satisfactory performance. The results are recorded in Table 3 in recognition of their possible applicability in other circumstances.

Benzene extracts of carbon black without further chemical treatment were found to be suitable for spotting on thin-layer cellulose plates for development and separation. A sample of furnace black weighing 297 grams (see Table 2) was extracted for several hours in a Soxhlet extractor with boiling benzene. The rather large volume of benzene was then reduced to less than 50 milliliters and made up to exactly 50 milliliters in a volumetric flask. A 5-microliter sample was then spotted on a thin-layer

TABLE 3

RESULTS OF ADSORBENT AND DEVELOPER COMBINATIONS

ADSORBENT	THICKNESS (Microns)	DEVELOPER PROPORTIONS	DEVELOPMENT TIME (Hours)	SAMPLE EXTRACTION PROCEDURE	RESULT
MN Cellulose 300	237	DMF:water 40:60	1.5	Benzene extract of carbon black	unsatisfactory
MN Cellulose 300	237	DMF:water 30:70	1.5	Benzene extract of carbon black	unsatisfactory
MN Cellulose 300	250	DMF:water 50:50	2.0	Benzene extract of carbon black	satisfactory 6 distinct zones
MN Cellulose 300	250	DMF:water 50:50	2.0	Benzene extract of rubber	unsatisfactory
MN Cellulose 300	250	DMF:water 50:50	2.0	Benzene extract of rubber:DMF solvent	satisfactory 6 distinct zones
Coarse filter paper Whatman's No. 50		DMF:water 50:50	overnight	Benzene extract of rubber: DMF solvent	unsatisfactory
Acetylated cellulose Series 40%-20%	250	Methanol- ether-water 4-4-1	2.0	Benzene extract of rubber: DMF solvent	unsatisfactory
Acetylated cellulose Series 40%-20%	250	Petroleum ether:methanol 99.25:0.15	0.3	Benzene extract of rubber: DMF solvent	unsatisfactory
Aluminum oxide G	250	Pentane:ether 19:1	0.28	Benzene extract of rubber: DMF solvent	unsatisfactory
Silica Gel G	250	Pentane:ether 19:1	0.28	Benzene extract of rubber: DMF solvent	unsatisfactory
Avicel SF	250	DMF:water 50:50	2.0	Benzene extract of rubber: DMF solvent	unsatisfactory

cellulose plate and developed in N,N-dimethylformamide and water. In-
spection under 3660-Angstrom ultraviolet light showed a separation of
fluorescent compounds into six distinct zones, as shown in Figure 2, which
were numbered bottom to top from 1 to 6. The apparent colors under ultra-
violet light are shown adjacent to the zones. These six zones were selec-
ted because of their prominence in all extracts of carbon black and carbon
black products studied. Their general arrangement after development is
shown in Figure 5. These zones, their colors, distances of migration,
and gradations into other zones are invaluable landmarks for the compari-
son of extracts from different sources. By varying the proportions of
the developer constituents, or by the use of other developers, it was
possible to obtain more zones than the six selected, but not in such
discrete areas.

The six distinct zones were harvested from a series of plates until
the concentration of polycyclics was great enough for the determination
of the ultraviolet absorption spectrum of each.

The relative positions of the comparable zones obtained from carbon
black, abraded rubber from old tires, and abraded new rubber from the
Bureau of Standards are shown in Figure 5. The fractions separated from
carbon black and from abraded aged rubber represent the same quantities
of carbon black. The fraction separated from the new rubber abraded by
the Bureau of Standards is much weaker because of the difficulty in ex-
tracting the polycyclic material from rubber which was mixed with the
alumina used as an abrasive. There was the further difficulty of cal-
culating the amount of carbon black represented since the weight of
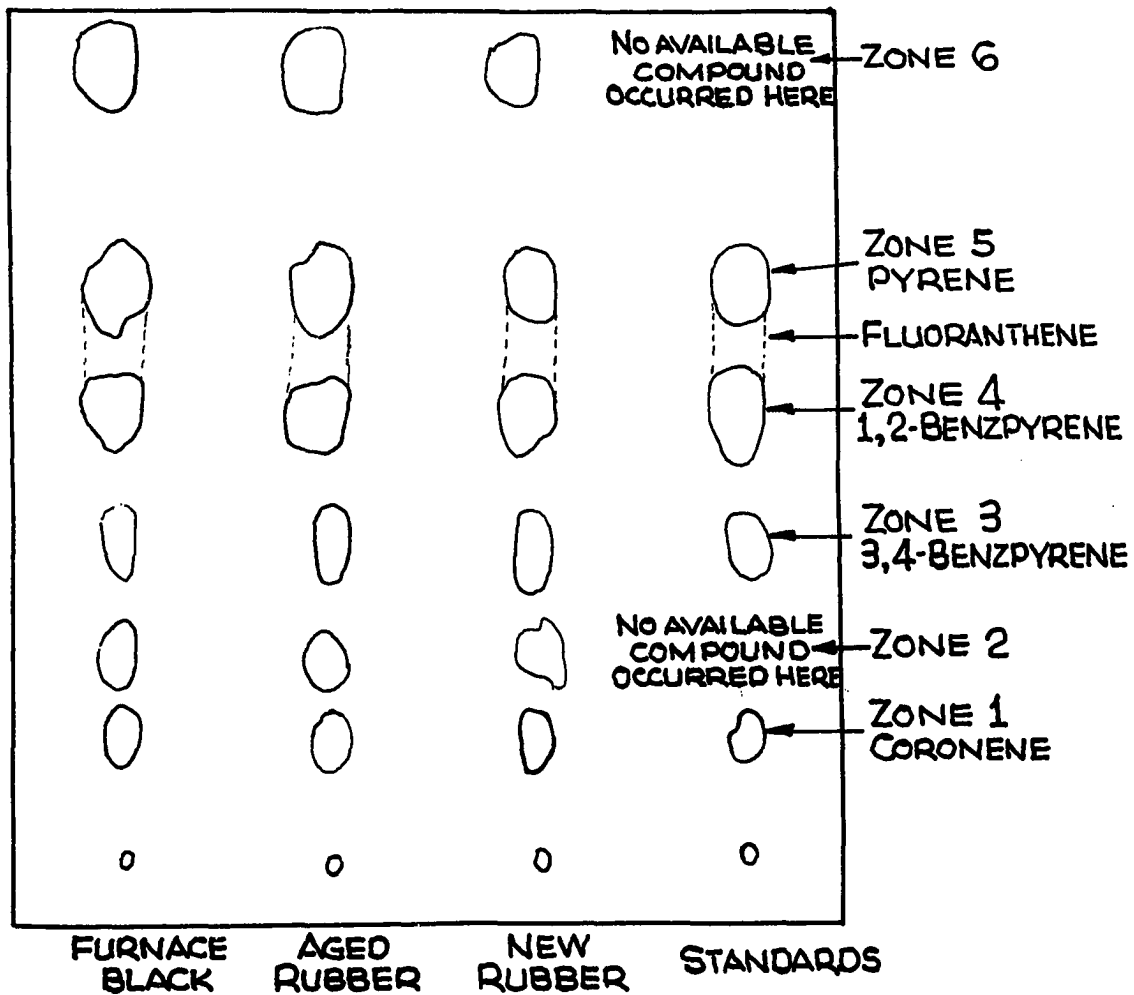abrasives mixed with the sample was not known. Though the abraded new

Figure 5. Parallel chromatograms of recovered products and standard compounds for comparison of colors, zones, and distances of zone migration.

rubber is not displayed on a quantitative basis, the assortment and
relative concentrations of the six zones are strikingly similar to the
six zones observed in the other two separations.

One unusual feature of the chromatograms exists in the position of
the frontal zones of both rubber extracts. In these two instances, the
position of the sixth zone lagged farther behind the solvent front than
was true in the case of corresponding products recovered from the carbon
black. The zones are of the same color and relative positions and their
absorption spectra are the same. The difference arises because some sub-
stance brought over from the rubber exerts its influence near the solvent
front. This is a situation which has been observed by other workers in
thin-layer chromatography.

By observing the assortment of polycyclics exhibited in the chromat-
ograms prepared from carbon black and from equivalent quantities of abraded
aged rubber as shown in Figure 5, it was concluded that there were few,
if any, differences in the number, kind, or concentration of the poly-
cyclics present. Partial evidence for this conclusion was forthcoming
from a comparison of the absorption spectra of the recovered products.

The total quantities of the polycyclics exhibited by chromatography
of the abraded new rubber were much less than those observed in the other
two materials. The number, species, order, and colors of the components
were strikingly parallel, however.

Each of the six zones observed on the thin-layer plates was believed
to consist of one or more polycyclic compounds. Though a mixture of sub-
stances was present, it was possible to gain some insight into the iden-
tity of the polycyclic compounds. Benzene solutions of coronene,

3,4-benzpyrene, 1,2-benzpyrene, fluoranthene, and pyrene were spotted in parallel development on the plates. The zones were then found to align in color and position with the following standards:

zone 1 with coronene

zone 2 with no standard available

zone 3 with 3,4-benzpyrene

zone 4 with 1,2-benzpyrene and fluoranthene

zone 5 with pyrene and fluoranthene

zone 6 with no standard available.

This is shown schematically in Figure 5.

It was therefore concluded that zones 1, 3, 4, and 5 probably contained coronene, 3,4-benzpyrene, 1,2-benzpyrene, fluoranthene and pyrene respectively, while zones 2 and 6 were unidentified. These conclusions except for zone 1 were supported by comparison of the absorption spectra of the products recovered from these zones.

The polycyclic compounds present in the furnace black furnished by the B. F. Goodrich Company were compared with those recovered from the carbon black present in autopsied monkey lungs. The results are diagramed in Figure 6. From a study of the chromatograms it was concluded that the B. F. Goodrich furnace black and the furnace black from monkey lungs were similar in that both contained 1,2-benzpyrene, pyrene, and possibly fluoranthene with two unidentified compounds present in each occurring at zones 2 and 6. The two carbon blacks were dissimilar in that furnace black from monkey lungs contained no coronene or 3,4-benzpyrene.

The chromatograms of the channel black from lung tissue appeared as

a single weakly fluorescent blue streak with no separation into distinct zones. Its general appearance is shown in Figure 6. This result may be attributable to the low concentration of polycyclic compounds noteworthy of some channel blacks. The presence of typical fluorescence in this instance, however, is almost certain evidence that some polycyclics were present. Yields of tissue polycyclics in this study were low because of the difficulty with emulsions. Because of the low concentrations available no absorption spectra could be obtained from the products recovered from lung tissue. This situation points up the value of fluorescence comparisons on thin-layer plates when polycyclics are present in very small quantities.

The absorption spectra of the polycyclics recovered at zone 1 from Goodrich furnace black, abraded aged rubber, and abraded new rubber are shown in Figure 7. The furnace black spectrum shows enough detail to indicate the presence of coronene, the absorption spectrum of which appears in Figure 7 as a very thin dashed line. The furnace black and coronene standard have common absorption maxima at 337, 332, 324, and 301 millimicrons. The spectra of the corresponding zones from aged and new rubber resemble the standard in only the most general features. Since the absorbance values of the carbon black product and the aged rubber product lie relatively close together it is surmised that no great difference in concentration exists, even though the spectrum of the rubber product is clouded by extraneous materials.

No standard polycyclic compound was available whose absorbance spectrum could be compared with that of the product from the second zone of the thin-layer plate. The absorbance spectra of the products
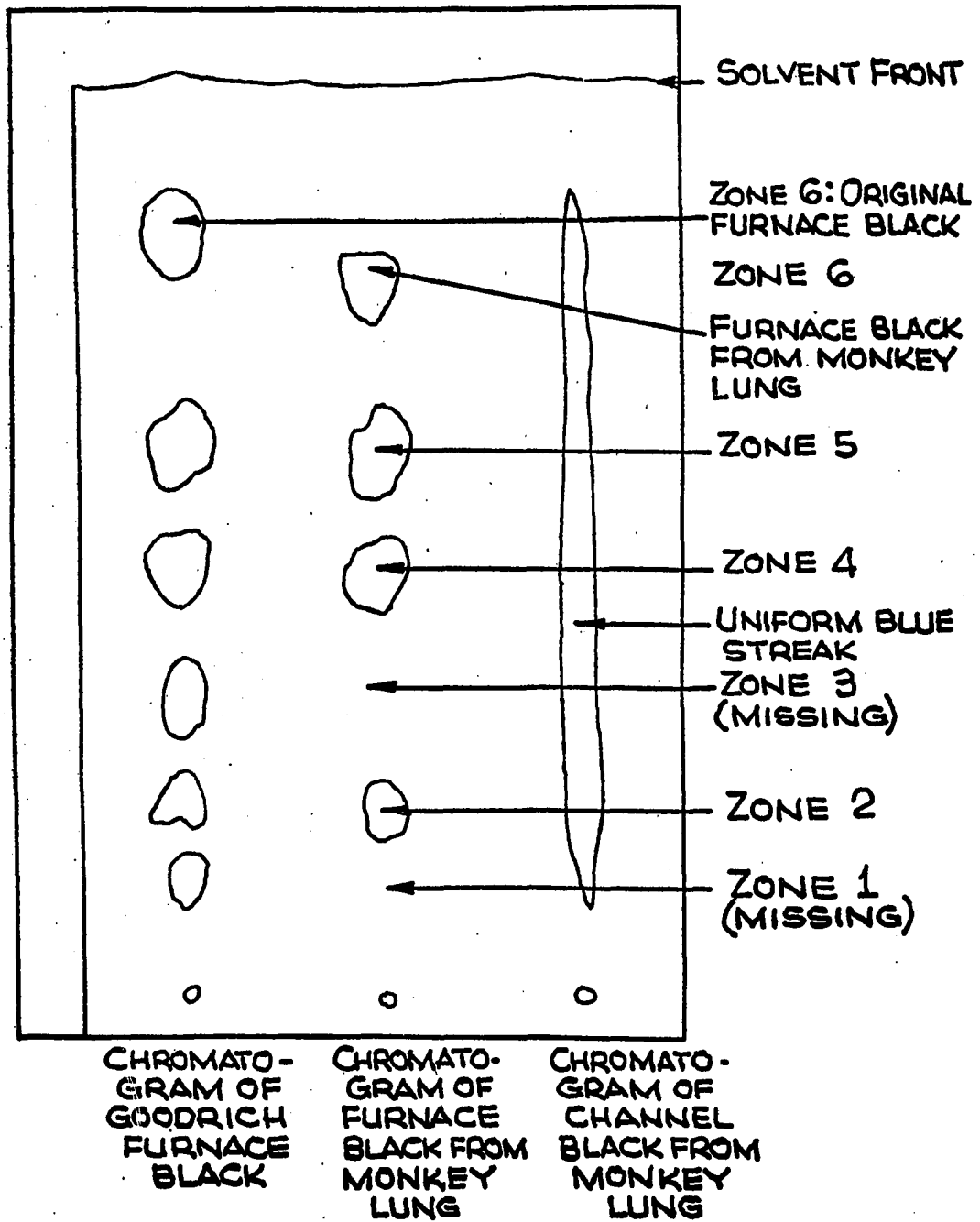
Figure 6. Comparison of the chromatograms of furnace black, furnace black from monkey lungs, and channel black from monkey lungs.
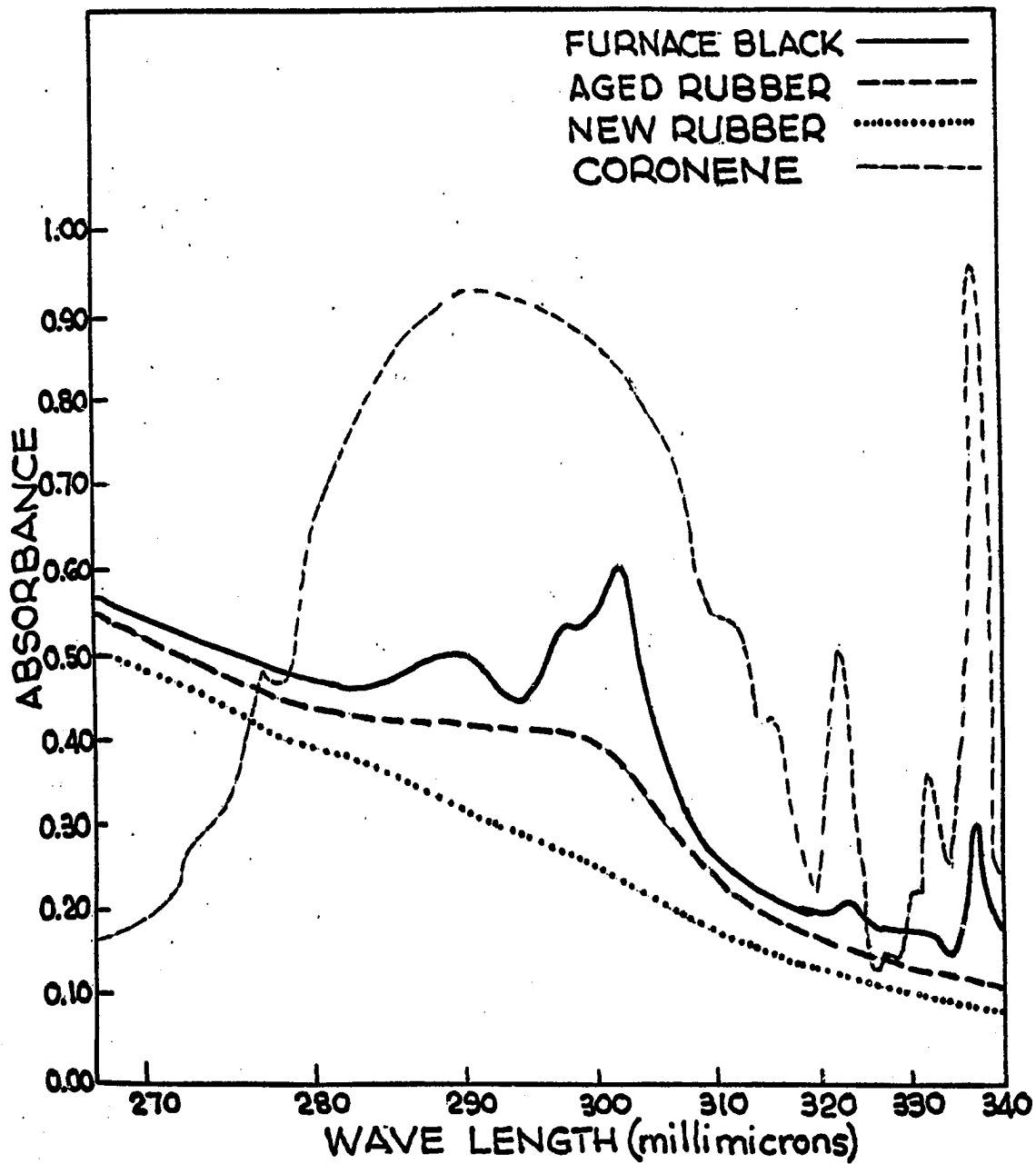
Figure 7. The absorption spectra of coronene and the products accumulating at the first zone.

recovered at this zone are exhibited in Figure 8. It is likely that more than one compound has accumulated here. The slight variation in position and color of this zone in repeated platings constitutes some argument for regarding this zone as encompassing more than one compound. Concentrations of this product in the free furnace black and in the furnace black incorporated in the rubber are believed to be very near the same. Some correction should be allowed for the several solvent system treatments necessary for the rubber. The absorption spectrum of zone 2 from the new rubber has been shifted upward along the absorbance scale in order that its maxima might be observed in juxtaposition with the comparable product.

A comparison of the absorption spectra of the products which accumulated at zone 3 is shown in Figure 9. The absorption maxima at 328, 315 and 282 millimicrons are such as to indicate the presence of 3,4-benzpyrene in the aged rubber. The absorption spectra of the recovered products are remarkably parallel though definition of detail is much less for the rubber products than for the furnace black.

The absorption spectra of the products from each of the three sources accumulated at zone 4 are shown in Figure 10. In Figures 10, 12, 14, 15, and 17 the discontinuity of the curves arises because some of the absorption bands occur at absorbance values greater than 1.0. The spectra of material from the carbon black and from aged rubber shown in Figure 10 are very similar both in concentration and in the location of absorption maxima; however, the absorption spectra obtained from the new rubber for this particular zone correspond to the other products only in the most general features. The standard
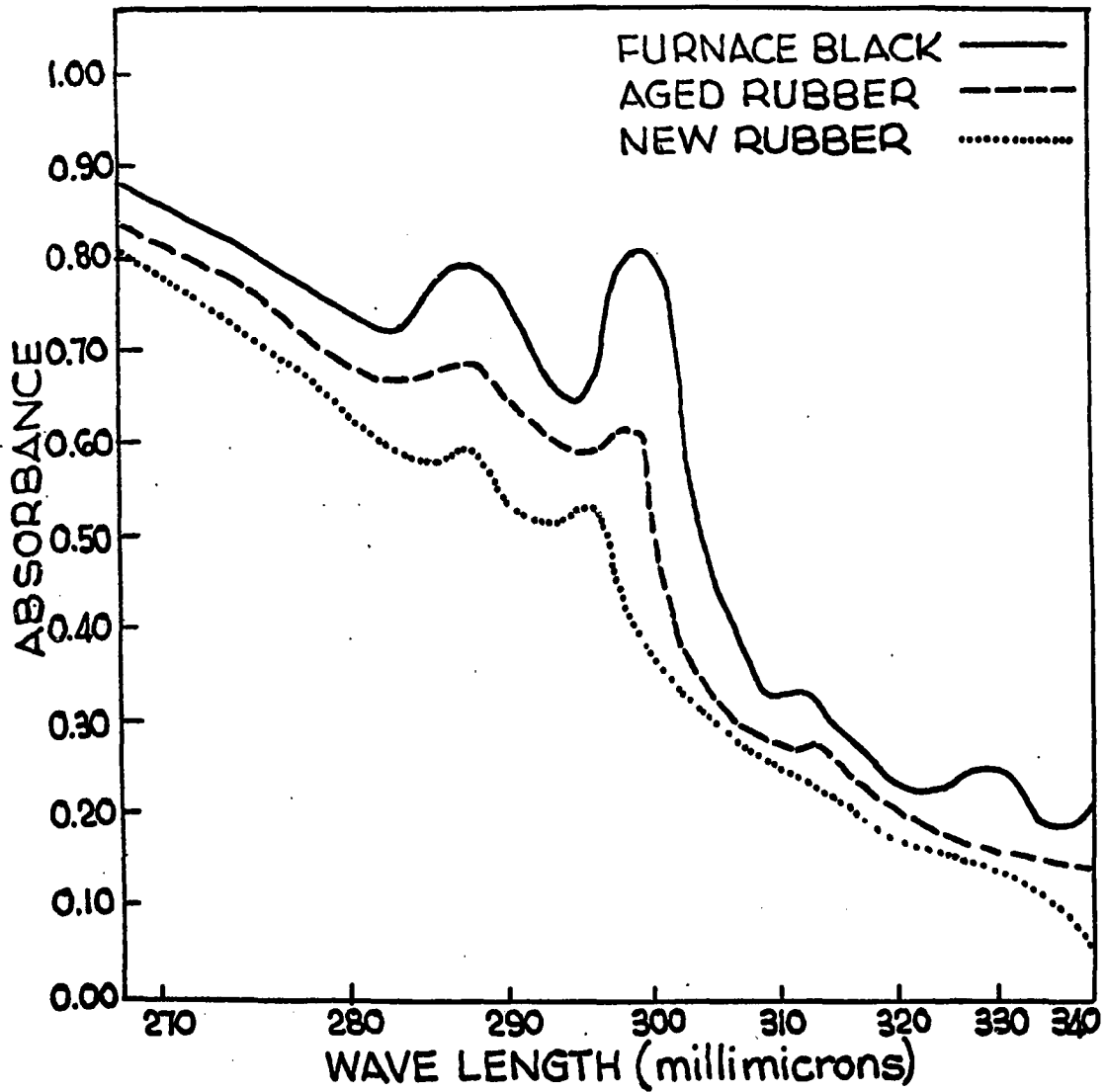
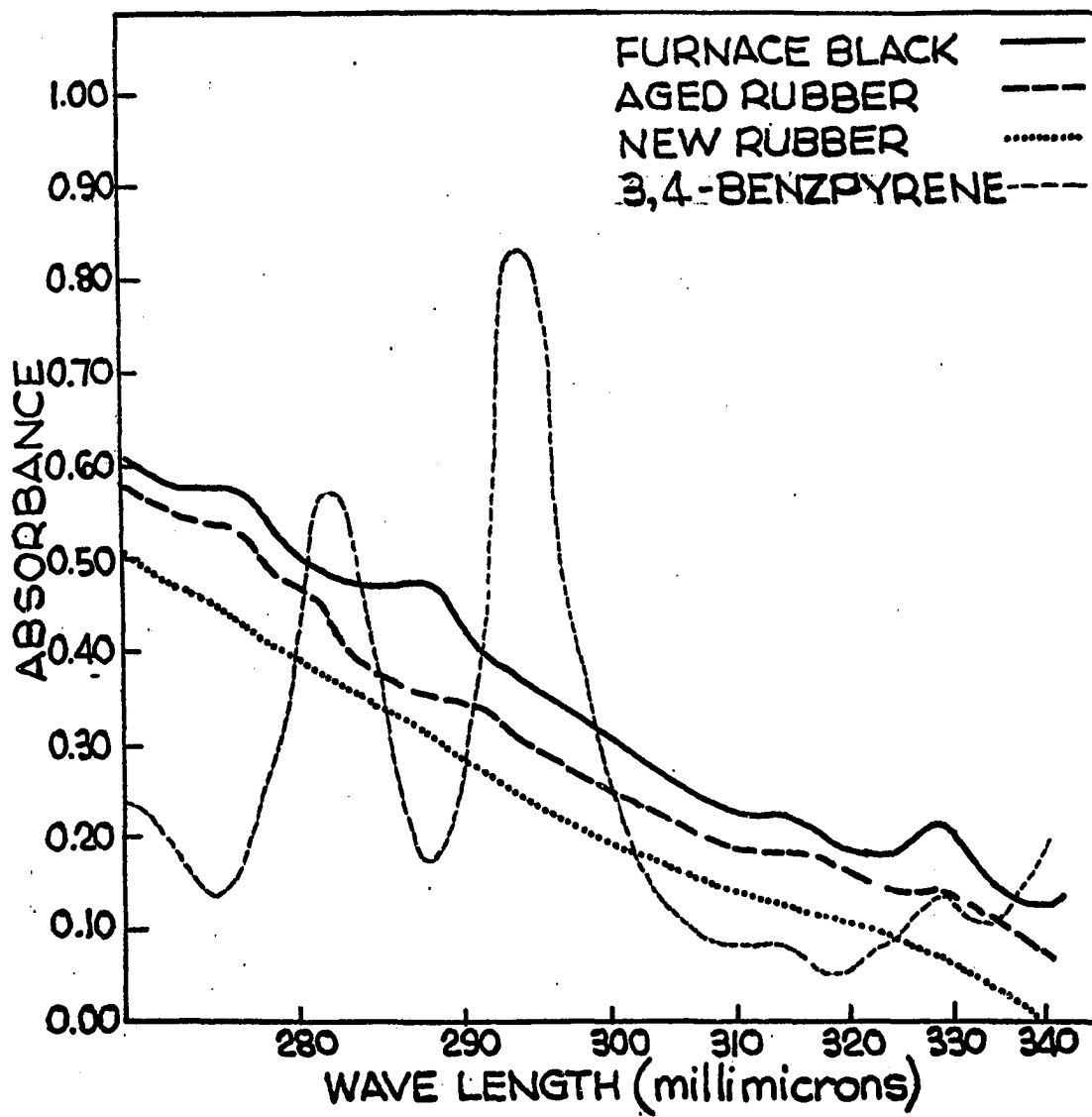Figure 8. The adsorption spectra of the products accumulating at the second zone.

Figure 9. The adsorption spectra of 3,4-benzpyrene and the
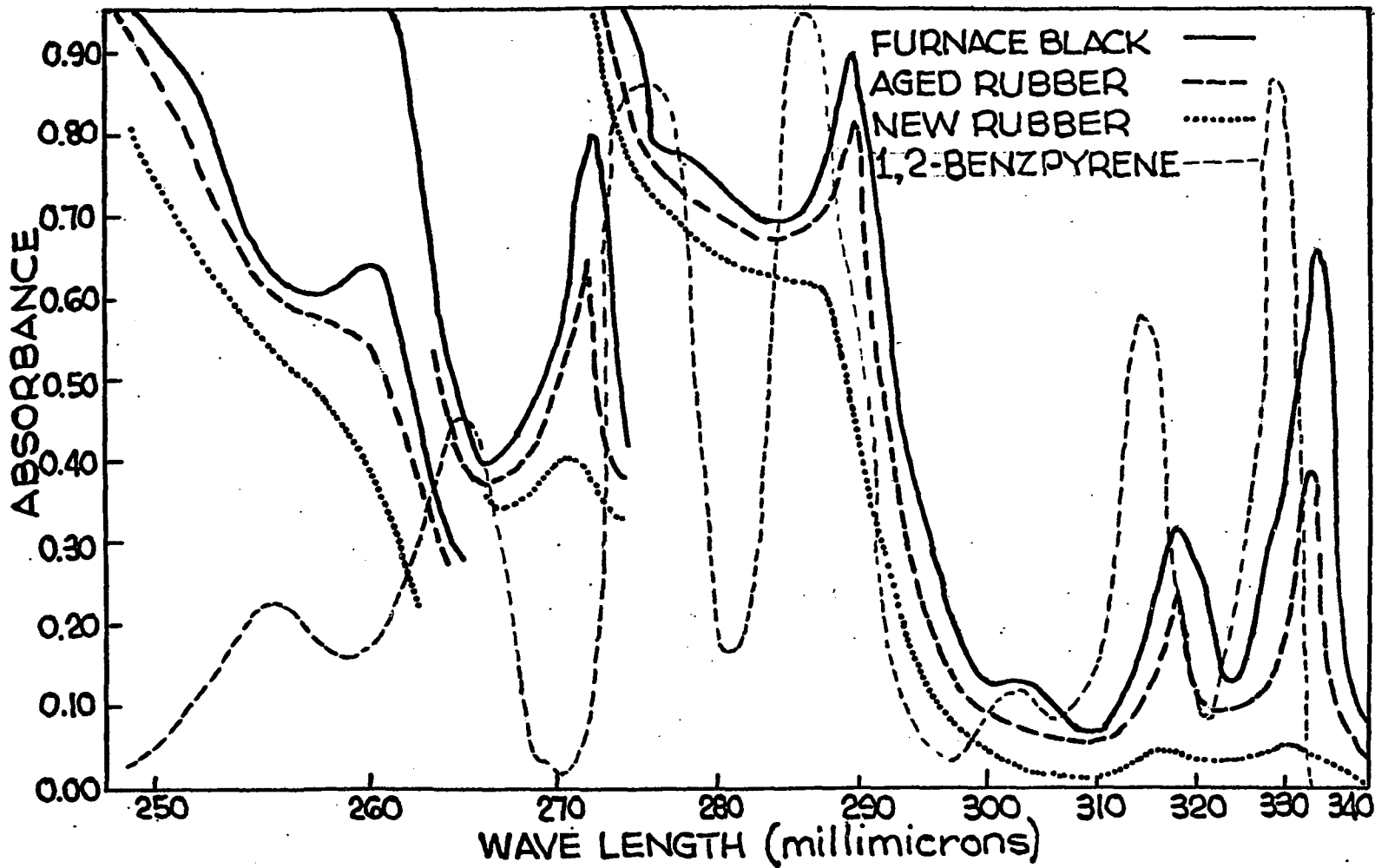products accumulating at the third zone.

Figure 10. The absorption spectra of 1,2-benzpyrene and the products accumulating at the fourth zone.

polycyclic compound aligning in this chromatographic zone is 1,2-benz-pyrene. Its spectrum, also shown in Figure 10, is much more sharply delineated than that obtained from the carbon black or rubber products. However the agreement of the spectra of the recovered products with the standard is such as to indicate that 1,2-benzpyrene is present.

The absorption spectra of the products which occurred at zone 5 are shown in Figure 11. The material from the furnace black and from the aged rubber correlate very closely in all details. Even the material from the new rubber has corresponding absorption maxima. The standard polycyclic compound appearing at this zone is pyrene and its absorption features are also shown in Figure 11. The intensity of the fluorescence of this zone and the heavy absorption bands indicate that pyrene is present in greater quantity than was true of any other polycyclic so far studied. The absorption spectra of these materials and standard pyrene are displayed in the wavelength range from 340 millimicrons to 270 millimicrons. Good absorption data were also obtained in the region from 270 to 230 millimicrons and the details are shown in Figure 12. The most striking detail is the occurrence of a shoulder at about 237 millimicrons after the maximum at 240 millimicrons. This configuration is common to both the furnace black product and that of the aged rubber. It is not exhibited in the corresponding position for the product from new rubber. There is a further anomaly in that this shoulder is only faintly suggested by the pyrene standard. This is some indication that still other polycyclics have accumulated at this region. These particular spectra will be discussed in the light of some comparisons which will be made about zone 6.
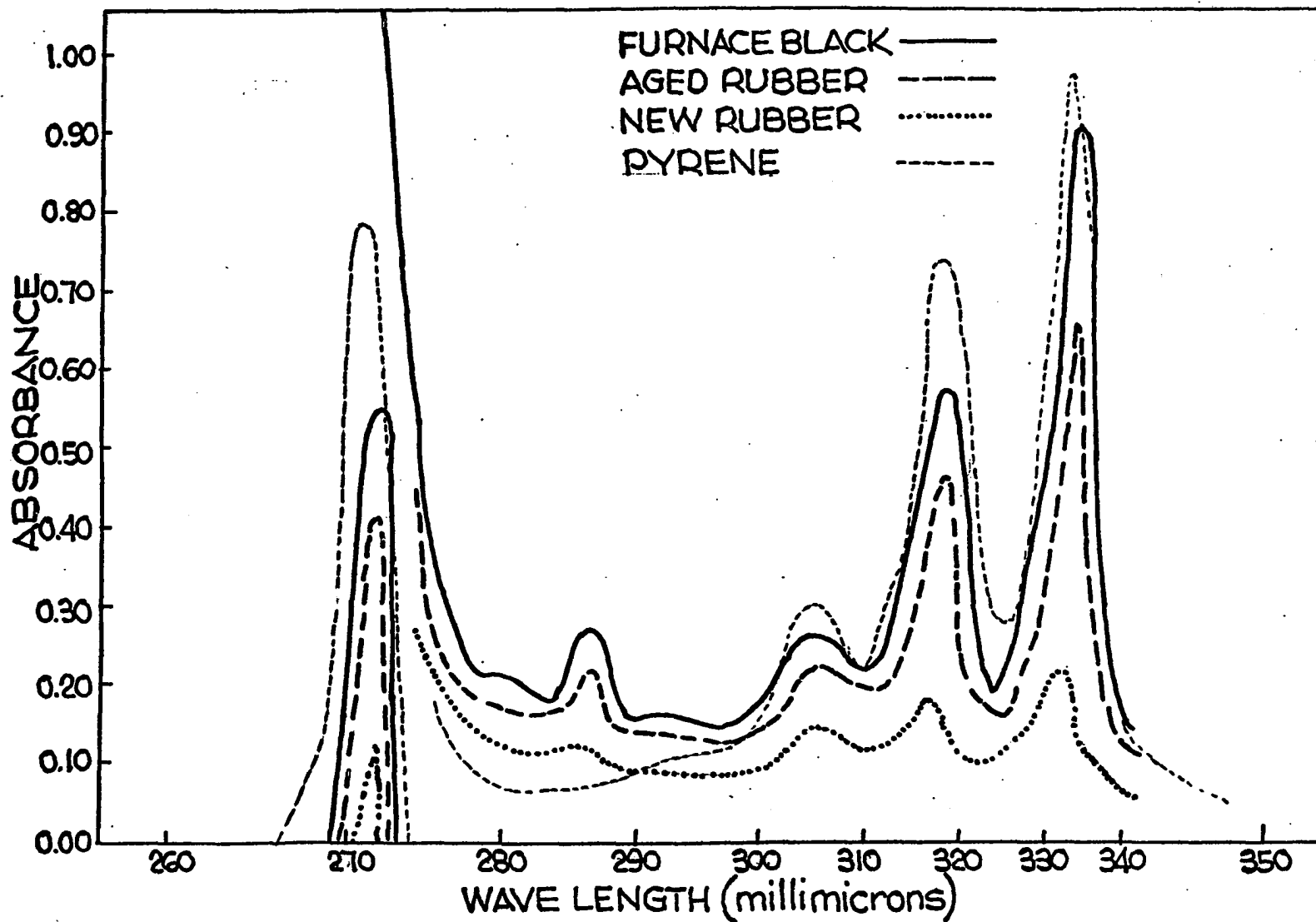
Figure 11. The absorption spectra of pyrene and the products accumulating at the fifth zone (340-265 millimicrons).
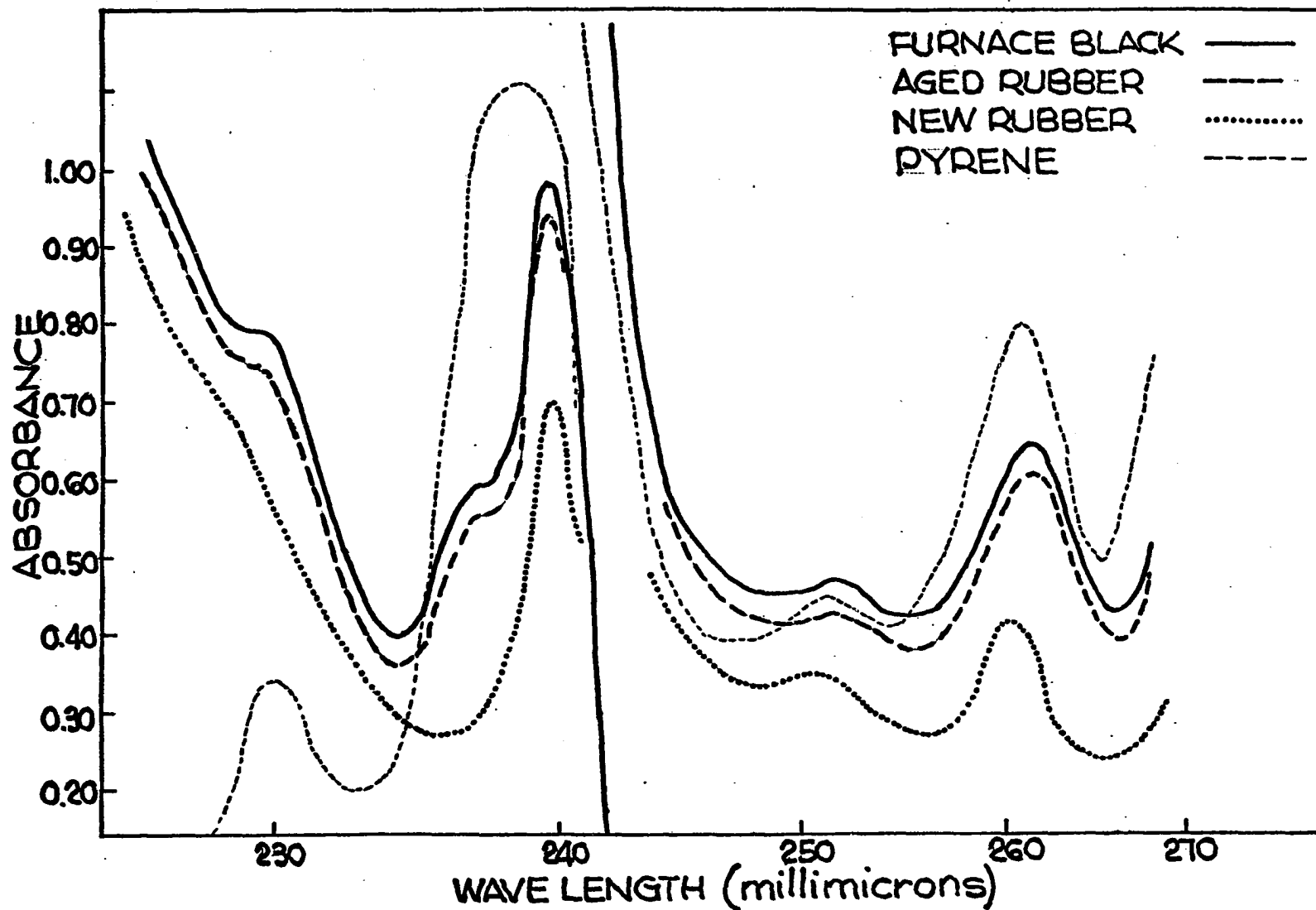
Figure 12. The absorption spectra of pyrene and the products accumulating at the fifth zone (265-225 millimicrons).

The products which accumulated at zone 6 were very striking for their brilliant purple fluorescence on the chromatographic plate. It could be surmised that a fluorescent product had accumulated there in very high concentration, but an examination of the corresponding absorption spectra shown in Figure 13 did not bear this out, at least in the range of 342-260 millimicrons. The spectra of the three products showed few distinct absorption bands though the general correspondence throughout the range was good. The absorbance values were such as to indicate the recovered products had occurred at about the same concentrations.

From the details of the standards shown in the thin-layer plates and displayed schematically in Figure 5, it would be expected that the polycyclic fluoranthene might appear in both zones 4 and 5. From the appearance of the cellulose plate, fluoranthene appears to concentrate in the area between zones 4 and 5. The absorption spectrum of fluoranthene in the 340 to 250 millimicron range is shown in Figure 14. The furnace black product from zone 4 is also shown in this figure in juxtaposition. The spectra resemble in some details which are anomalous. It may be assumed that the peak observed in the carbon black extracts at about 317 millimicrons corresponds to the peak in the standard at 320 millimicrons, with the latter being shifted to the higher wavelength. This is a comfortable assumption until observation of the peaks at 289 where the standard is shifted toward the lower wavelength. The peaks at 260 millimicrons are in agreement except that for fluoranthene no really sharp maximum is defined. This situation indicates the presence of a mixture in the case of the carbon black product and suggests that fluoranthene is a component.
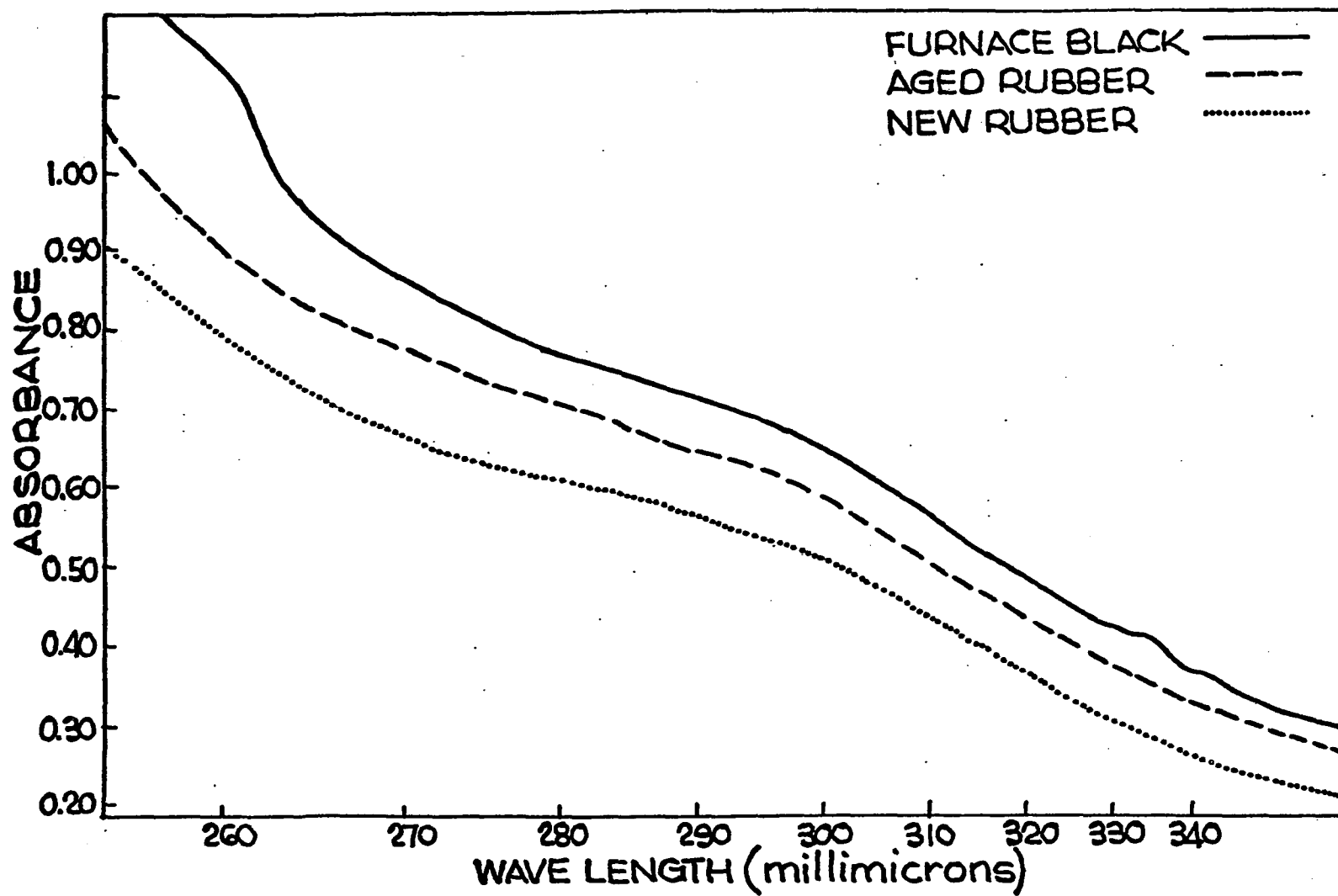
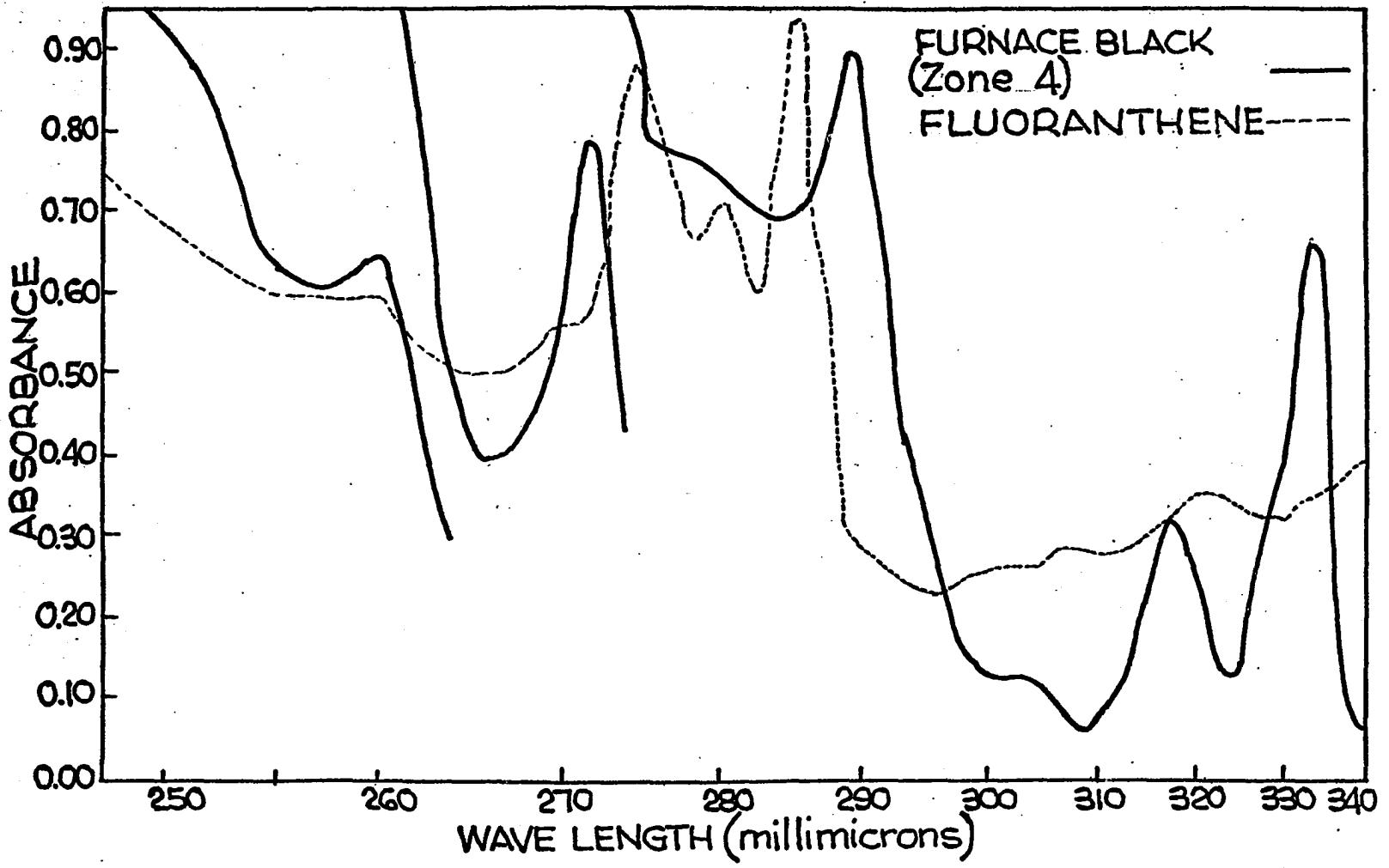Figure 13. The absorption spectra of the products accumulating at the sixth zone.

Figure 14. The absorption spectra of fluoranthene and the furnace black products accumulating at the fourth zone.
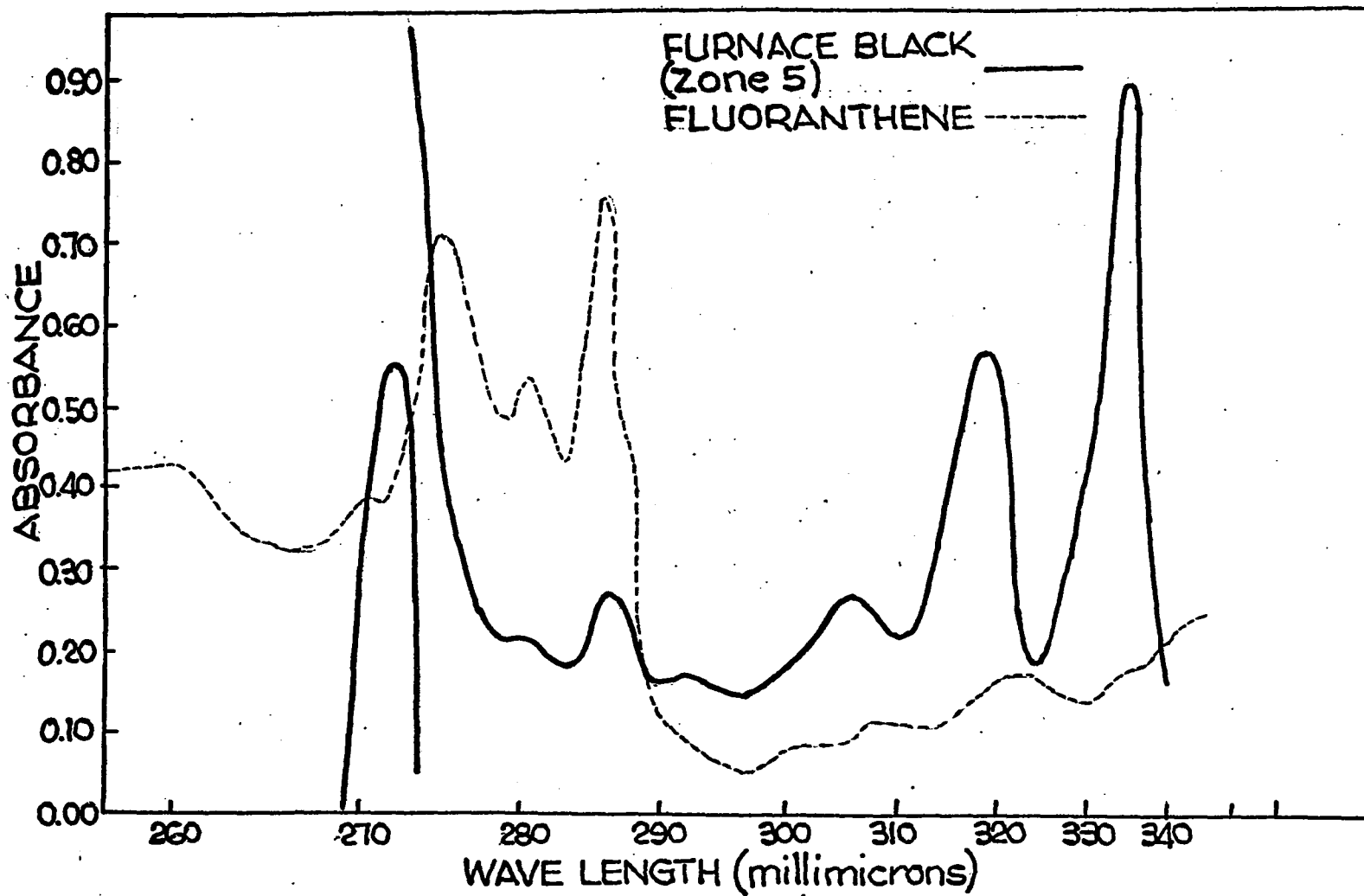
Figure 15. The absorption spectra of fluoranthene and the furnace black products accumulating at the fifth zone.

Figure 15 is a comparison of the spectrum of the furnace black

product of zone 5 with that of the standard fluoranthene over the same

wavelength range. The absorbance similarities between 270 millimicrons

and 287 millimicrons are noteworthy. The occurrence of similar absorp-

tion bands and the position and color of the products on the plate are

such as to suggest that fluoranthene was a component of the furnace

black product concentrating at zone 5.

Some of the details of the molecular structures of pyrene, 1,2-benz-

pyrene, and 3,4-benzpyrene are shown in Figure 16. There is a grouping

of four of the rings as a nucleus which is common to all three compounds.

| Pyrene | 1,2-benzpyrene | 3,4-benzpyrene |
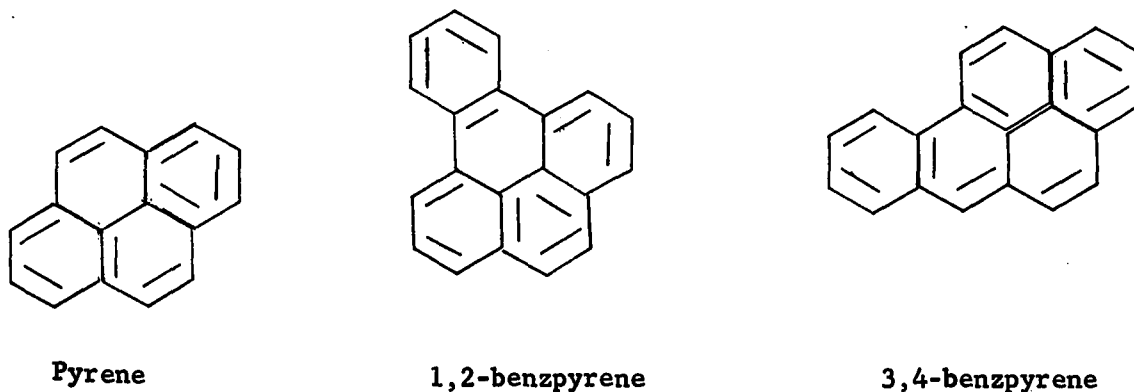
Figure 16.   Some details of the molecular structure of pyrene,
1,2-benzpyrene, and 3,4-benzpyrene.

Despite this structural similarity, the ultraviolet absorption spectra of

1,2-benzpyrene and 3,4-benzpyrene are distinctly different as is indicated

in Figure 17. It is, therefore, not likely that these two compounds would

be confused where instrumentation is available for displaying their respec-

tive absorption spectra. Also displayed in Figure 17 is the absorption
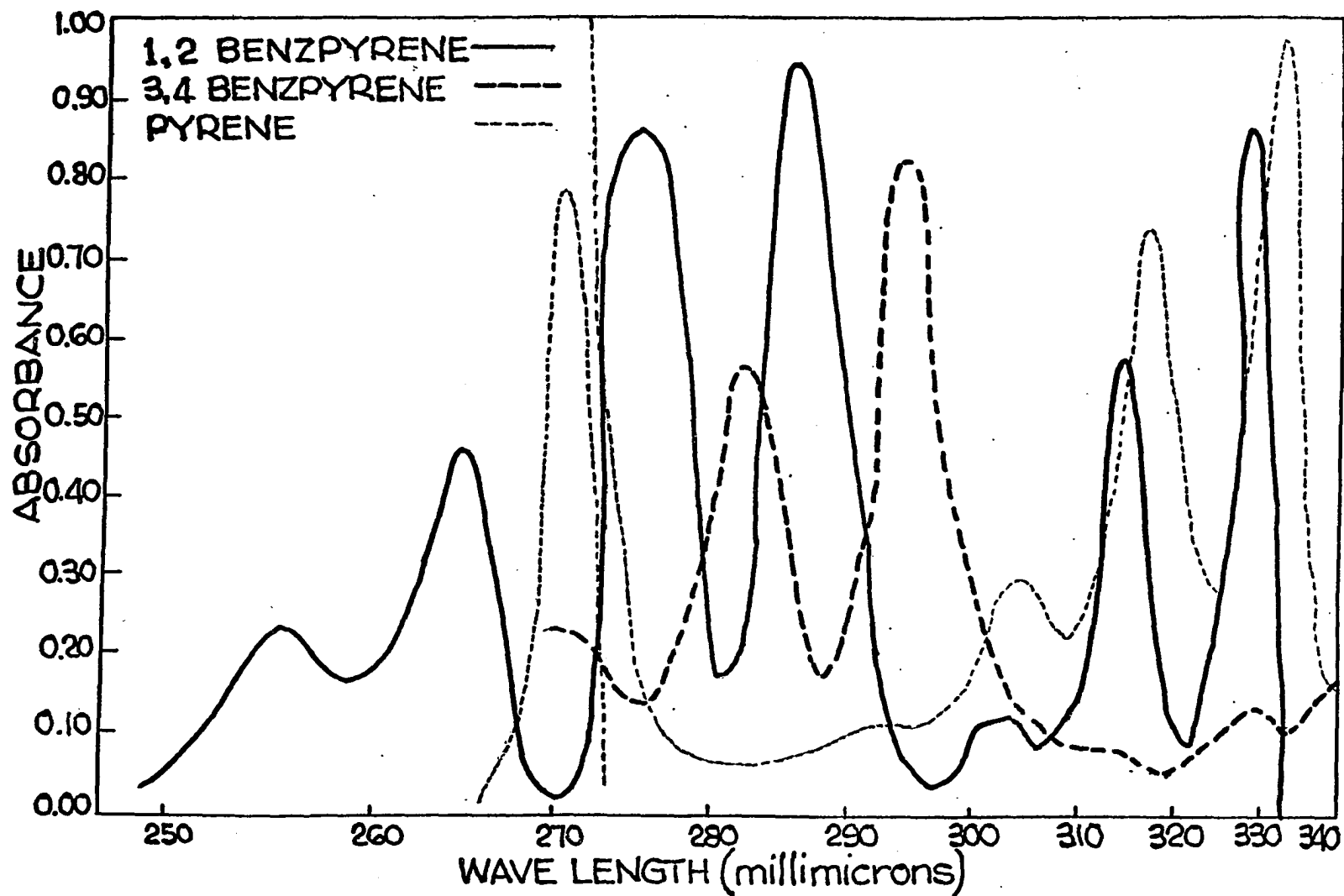
Figure 17. The absorption spectra of 1,2-benzpyrene, 3,4-benzpyrene, and pyrene.

spectrum of pyrene. The occurrence of three approximately corresponding maxima in the 240-295-millimicron region is a suggestion of the parent nucleus present in pyrene and in 1,2-benzpyrene. Why these three maxima are so ill-defined in the case of 3,4-benzpyrene is another property perhaps as inscrutable as the very strong carcinogenic character of that compound.

CHAPTER VII

SUMMARY AND CONCLUSIONS

Vehicular tire rubber is being abraded into the environment in ever increasing quantities to become an air pollutant and to accumulate in soil and water. The presence of polycyclic aromatic hydrocarbons in carbon black used in the processing of rubber makes abrasions from vehicular tires a possible source of carcinogens. The purpose of this inquiry was to develop a method for conveniently determining the presence of polycyclic aromatic hydrocarbons in carbon black and vehicular tire rubber and to assess the influence of processing, age, and abrasion on the assortment and concentration of these compounds. The procedure developed for achieving this purpose was as follows:

a) The starting material, either furnace black or abraded vehicular tire rubber, was extracted for 24 hours with hot benzene in a Soxhlet extractor. If the extracted material was furnace black, its extract, after reduction in volume, was ready for spotting on thin-layer chromatograph plates. If the extract was of rubber, it was necessary to proceed through the following solvent systems:

1) The benzene extract of rubber was reduced in volume and taken up in a small quantity of isooctane to be extracted 3 times

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successively with small portions of 80 per cent

N,N-dimethylformamide in water.

2) The accumulated N,N-dimethylformamide was then extracted

with a mixture of isooctane and water and the water was

discarded.

3) The isooctane was dried with anhydrous sodium sulfate and

adjusted with additional isooctane to the original volume

of benzene.

b) The extract, in benzene or isooctane, was reduced in volume,

spotted on a thin-layer chromatographic plate of the cellulose

called MN300 by Brinkman Instruments of Westbury, New York, and

ascendingly developed for 2 hours with a mixture of N,N-dimethyl-

formamide and water in equal proportions.

c) The separated spots were made fluorescent under 3660-Angstrom

ultraviolet light to be delineated and identified by comparison

with parallel chromatograms of known polycyclic compounds.

d) The separated spots were harvested, dissolved in hot methanol

and their absorption spectra determined.

e) The identity of the recovered products was confirmed by compari-

son of the absorption spectra with that of known compounds. If

a product could not be identified, its absorption spectrum and

its plate position were used to establish its presence and

relative concentration in each of the materials studied.

By the use of this procedure B. F. Goodrich furnace black, abraded

new vehicular tire rubber and abraded old vehicular tire rubber were

analyzed for the presence of polycyclic aromatic hydrocarbon compounds.

By thin layer chromatography it was suggested that each of these materials contained coronene, 3,4-benzpyrene, fluoranthene, 1,2-benzpyrene and pyrene. Ultraviolet absorption techniques, however, supported only the presence of pyrene and 1,2-benzpyrene in these three samples. From a calculation of the amount of furnace black used in the vehicular tire rubber, it was established that processes of manufacture, aging and wear had not reduced the assortment of polycyclics as evidenced by thin layer plates or the concentration in instances where absorption data could be obtained. This means that polycyclic compounds continue to exist in abraded vehicular rubber of the age employed in this investigation.

A chromatogram of the channel black showed a uniform fluorescent streak indicative of the presence in low concentration of polycyclic compounds. Their identity could not be established.

The thin layer plates indicate the furnace black from monkey lungs to be similar to the B. F. Goodrich furnace black in that both yield zone positions and colors corresponding to 1,2-benzpyrene, pyrene, fluoranthene and two unidentified compounds occurring at zones 2 and 6. The two carbon blacks were dissimilar in that furnace black from monkey lungs contained no coronene or 3,4-benzpyrene. The absence of these compounds could be explained by assuming one of the following alternatives:

1) They were absent from the furnace black inhaled.

2) They were altered or were not detected by the analytical procedures employed.

3) They were present when the furnace black was inhaled but were eluted and carried away by physiological processes.

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