
L. M. SHABAD

Institute of Experimental and Clinical Oncology of the Academy of Medical Sciences of U.S.S.R., Moscow, U.S.S.R.

SUMMARY

The role of the retention of carcinogenic hydrocarbons in tissues in carcinogenesis was demonstrated. Possible contamination of food with carcinogenic hydrocarbons is discussed and the measures for its prevention are planned. Much attention was given to the study of the exhaust gases of cars and to the prevention of their carcinogenic action. The distribution of benz(a)-pyrene in human environment and the fate of this substance were traced. Soil pollution with benz(a)pyrene is described; its destruction by some soil microorganisms is demonstrated.

INTRODUCTION

The quantitative determination of carcinogenic substances plays an eminent part both in the study of cancer pathogenesis and in cancer prevention. Hydrocarbons and especially benz(a)-pyrene [3,4-benzpyrene, (BP)] occupy a particular place among carcinogenic substances. They were the first to be discovered and are perhaps the most investigated. Their presence in the human environment is clearly confirmed by direct detection for example in air pollution. Carcinogenic hydrocarbons undoubtedly can induce cancer not only in experimental animals but also in human beings, as they constitute active agents of different tars, mineral oils, pitches, soots, etc., causing such well-known occupational tumors as the classic chimney-sweep's cancer, the briquette-maker's and mule-spinner's cancer, etc. Owing to their property of luminescence under the influence of ultraviolet light they can be rather easily detected in complex mixtures, and finally it must be emphasized that carcinogenic hydrocarbons do not represent natural substances, but are the products of human activity, and for this reason they can and must also be rendered harmless by human activity.

Spectrofluorescent methods of determining carcinogenic hydrocarbons have been considerably improved in connection with the discovery of the so-called Shpolsky's phenomenon made in the U.S.S.R. The latter consists of obtaining distinct, almost linear, individual luminescence spectra of a number of complex organic molecules under low temperatures (−196°C) in solutions of normal paraffins, instead of diffuse spectra obtained in normal conditions (33). This phenomenon served as a basis for several methods of quantitative determination of BP (8, 13, 14, 17, 18).

This paper is a short report on some investigations performed in our laboratory during recent years for the purpose of the quantitative spectrofluorescent assay of carcinogenic hydrocarbons and especially BP. This substance can be detected in such low concentrations as 1 × 10⁻⁶ mg/ml. In the products containing BP other hydrocarbons may also be present, but in smaller quantities. For special tasks spectrofluorescent methods of analysis can be worked out for hydrocarbons other than BP. For instance Danil'tzeva-Fedoseeva and Khesina (7) recently developed a method of quantitative determination of the strongest carcinogenic hydrocarbon, 7,12-dimethylbenz(a)anthracene (DMBA).

DISCUSSION

The Role of the Retention of Carcinogenic Hydrocarbons in Tissues

In our laboratory spectrofluorescent methods have been used for many years to study the fate of carcinogenic hydrocarbons in the organism, their distribution in various organs, and the mechanisms of their action. In 1961–1962 we (22, 26) succeeded in the elaboration of an experimental model of lung cancer (in 30–70% of cases) by means of intratracheal intubation of carcinogenic hydrocarbons (DMBA and BP) in rats. Comparison of the positive results with those of our previous experiments and with the data obtained by other authors (almost always negative) made possible the supposition that lung cancer induction depended on deposition and retention of carcinogenic hydrocarbons in lung tissue, due to their injection together with such adsorbents as black ink and casein. This assumption was later confirmed by the experiments of Saffiotti et al. (24) who produced experimental lung cancer in hamsters by the intratracheal injection of BP with hematite powder. The accumulation of carcinogenic hydrocarbons in lung tissue when injected with adsorbents was proved by spectrophotometric analysis of extracts from this tissue (31). Later, Pylev (23) in his experiments showed the importance of the adsorbent particle size and of various kinds of soot in particular: fine particles of soot retain carcinogenic hydrocarbons better than coarse ones. Thus the deposition of carcinogenic hydrocarbons in lung tissue may depend on many factors including very complicated relations between particles of different substances that can contribute to the accumulation of carcinogenic substances in lung tissue.

Andrianov (1) worked out an interesting technic of quantitative determination...
estimation by recording the elimination of minute doses of BP (0.01–0.1 μg) after its application to the skin of living mice. It was found that after each repeated application of this substance its elimination from the skin was retarded. As a result the effectiveness of each single dose increased with the number of applications, i.e., here too, the deposition factor plays an important part.

In the skin irradiated by β-rays of 144Ce, the BP resorption was also retarded. This delay may be responsible for the considerable increase in incidence of tumors induced by DBMA and BP in pre-irradiated skin as compared to the skin of non-irradiated animals (3, 34). Thus the intensification of the carcinogenic effect due to the combined action of radiation and chemical carcinogenic compounds may be explained not by their synergism or by certain kinds of co-carcinogenesis but by the deposition and accumulation of carcinogenic hydrocarbons in the skin.

At present, by means of spectrofluorescent methods and luminescent microscopy, we can study the possibility of absorption and transformation of BP by the cells of both normal tissues and tumors cultivated in vitro (2). Thus, explanted normal fibroblasts, which are known to be more sensitive to the toxic action of BP (36) seem to transform this substance more intensively than the cells of induced sarcomas, which are resistant to the toxic effect of BP.

The above data show the importance of accurate quantitative determination of carcinogenic hydrocarbons for the investigation of the mechanism of carcinogenesis. In addition, the determination of their minimal effective dosage should be taken into account when considering such important practical problems as the elaboration of minimal permissible doses or the concentration of carcinogenic hydrocarbons. At present such minimal permissible doses cannot be established. It is necessary to continue studying the mechanism of action of each single carcinogenic substance and to work out the methods of detecting the sources of these substances and the manner in which they enter the human environment.

Possible Food Contamination by Carcinogenic Hydrocarbons

Some years ago we established the presence of BP in smoked food (25). In different kinds of smoked fish and sausage quantities ranging from 2 to 10 μg per kg of BP (9, 10) were found. People who consume much smoked food, e.g., some groups of fishermen and workers in meat-smoking and fish-smoking factories (12, 37) have particularly high incidence of digestive cancer. For this reason technology of food smoking was altered, using special smoking liquids, which are free of BP and do not induce tumors when tested on animals (10, 21).

The problem of the presence of carcinogenic hydrocarbons in foodstuffs is not confined to smoked products. Recently we (28) studied a great number of refined solid paraffins used in the food industry. As a consequence, certain paraffins containing minimal quantities of BP were selected and purified, which made it possible to obtain paraffins fully free of BP.

At present cattle are fed with hydrolyzed yeast grown in a special cellulose medium. Our studies (29) showed that hydrolyzed yeast contains 10–20 μg of BP per kg of dry weight. This highly unexpected finding was substantiated in further investigations. It was found that among the salts added in considerable quantities to nutrient medium for cultivating hydrolyzed yeast, technical ammonium sulfate was used which is the product of the treatment of coal gas with sulfuric acid in the pitch-coke industry. According to our investigations this technical ammonium sulfate contained 1000–1400 μg of BP per kg.

The fate of this pollution is very interesting. In active silt samples obtained from the sewage (in sedimentation tanks) from a number of hydrolysis plants, we discovered BP in quantities varying from 30 to 140 μg per kg of silt. Active silt is also widely used in agriculture.

Detection of BP in products which may be used as fodder raises the question of its possible further transmission into food products used by man. Today we have no data confirming or denying such a possibility. The important measure of preventing carcinogenic contamination of foodstuff by the elimination of carcinogenic hydrocarbons from raw materials, was to recommend the substitution of the technical ammonium sulfate contaminated with BP.

Air Pollution and Exhaust Gases of Automobiles

For a number of years we have been studying the problem of BP air pollution in the cities of the U.S.S.R. We shall not dwell here upon the results of these investigations summarized in our book (27) but it must be emphasized that in those days our attention was concentrated primarily on the exhausts of industrial and heating systems. At present the motor vehicle is considered to be one of the main sources of air pollution.

In collaboration with the laboratory of car neutralizers (Chief, Professor I. L. Varshavsky) we recently conducted a number of quantitative assessments of the content of BP in exhaust gases of the combustion engine. This cooperative work aims at finding ways to reduce markedly the amounts of carcinogenic substances released by car engines into the atmosphere. The role of the motor vehicle in polluting modern cities with carcinogenic hydrocarbons may be illustrated by the following example: in soil samples collected near the sidewalk in one of the Moscow streets (one-way traffic, about 500 cars per hour) we found 21,150 μg of BP per kg of soil, while in the control sample taken from the soil of a nursery yard situated at a distance of several dozen meters from the same thoroughfare there were only 430 μg of BP per kg of soil. It is worth noting that 500 μg of BP per kg of soil corresponds approximately to the mean “background” of the pollution of Moscow soil with this substance in districts where there are no other sources of carcinogenic hydrocarbons (see below).

The BP concentration in air pollution was studied by Zabezhinsky (38) in Leningrad by an aspiration method in the streets with intensive automobile traffic with control samples taken in neighboring squares. The BP concentration was 0.74–6.9 μg per 100 cu m which was 2–4 times the background concentration. The BP content in air pollution on a highway changes during the day according to the level of the auto traffic, with some delay in time.

The great effect on air pollution by carcinogenic substances from motor vehicles may be easily understood in view of the fact that one diesel engine in starting and stopping one hundred times discharges about 500 μg of BP (35).

The greatest amounts of this carcinogen are produced during the deceleration cycle, i.e., near traffic lights, in places of traffic jams, etc. Thus it can be seen from the work of Zabezhinsky (38)
TABLE 1
Pollution of Soil in Different Districts of Moscow and the Suburbs by Benz(a)pyrene

<table>
<thead>
<tr>
<th>District under investigation</th>
<th>Content of benz(a)pyrene in soil (μg per kg of dry soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum</td>
</tr>
<tr>
<td>Oil-refinery</td>
<td>220,000.0</td>
</tr>
<tr>
<td>Asphalt-concrete plant</td>
<td>520.0</td>
</tr>
<tr>
<td>Old buildings</td>
<td>496.0</td>
</tr>
<tr>
<td>New housing</td>
<td>478.5</td>
</tr>
<tr>
<td>Settlement of Birulevo near Moscow</td>
<td>114.6</td>
</tr>
<tr>
<td>Field near Moscow*</td>
<td>99.4</td>
</tr>
<tr>
<td>Zone of rest on the Kljasma water storage reservoir</td>
<td>0</td>
</tr>
</tbody>
</table>

* Close to a thoroughfare with heavy traffic.

that in Leningrad the amount of BP at crossroads is 2.5 times as much as that found in the middle of the street.

While riding along one of the main Moscow highways in a stream of cars a Volga car exhausts 100 μg of BP during a 3-hour period. With the density of traffic equal to 500 cars per hour and providing that on an average one car discharges 1 μg BP per minute near a traffic light, about 20,000 μg of this substance (i.e. about 20 mg) are released within 24 hours.

According to the data of Zabezhinsky concerning the content of BP in the air (6.4 μg per 100 cu m), it is easy to calculate the approximate portion of carcinogenic substance that can be inhaled by a man during a day near the “stop” line in one of the Leningrad streets. It corresponds to about 2 μg of BP adsorbed on dust and soot particles.

It must be kept in mind that BP exhausted by cars, in contrast to that discharged by industrial plants and heating systems, pollutes the air on the level of man's and especially children's respiration. Such examples are numerous. Therefore it is evident that devices for the substantial reduction of carcinogenic hydrocarbons in car exhausts are of great importance. One of the means of reducing the amount of carcinogenic substances in car exhaust gases is the improvement of fuel combustion. This may be achieved for instance by the so-called forechamber-torch ignition. As has been shown by our investigations (40) forechamber-torch ignition can actually reduce the amount of BP discharged into the air by 15-20 times.

Another means is by the additional combustion of exhaust gases and the introduction of various neutralizers. Our investigations enabled us to work out methods of both selecting samples in different stages of engine work and with various neutralizers, and methods of accurate quantitative determination of BP in exhaust gases, and determined the quantities of exhausted BP as a function of engine work (starting and stopping the engine, working regime, idle run, etc.) (35). The continuation of investigations and comparison of the results of applying various neutralizers will give the opportunity to choose a number of the most effective models. At present one of the urgent problems in the control of air pollution and diminishing carcinogenic danger in cities is the necessity for collaborative work between engineers and oncologists on creating devices that prevent the air from being polluted with carcinogenic hydrocarbons, on the one hand, and of providing every car with such devices, on the other hand.

Soil Pollution by Benz(a)pyrene

Investigation of air pollution has shown that dozens or even hundreds of tons of dust fall out each year on the territory of modern large industrial cities. Taking into consideration the concentration of BP in air pollution one may say that during a year several dozen kilograms of BP may fall out on such a city. Naturally, the question arises regarding the further spread and fate of these carcinogenic hydrocarbons.

In this connection the study of BP content in soil becomes urgent. Only a few papers on this problem have been published so far (4-6, 15, 27, 39). Our laboratory (30, 32) has carried out investigations on soil benzene extracts, collected in various districts of Moscow and the suburbs (Table 1). In the control sample taken in the vicinity of the Kljasma water storage reservoir no BP was found, while within the city borders soil was highly polluted. Even in those districts of Moscow where the effect of large industrial plants seems to be actually negligible (e.g. a hospital yard), the concentration of BP was rather high—500 μg per kg of soil. This can be explained by the assumption that many sources, such as heating furnaces, city motor vehicles, etc., may be equally responsible for the pollution of the environment with carcinogenic hydrocarbons. Further, as we found in 1959 while investigating air pollution in Leningrad, the spreading pollution, especially of BP, from the source of discharge is so wide that several zones of pollution may fuse together, thus subjecting the population of the entire city area to the danger of contamination and to the carcinogenic hazard. Carcinogenic hydrocarbons are adsorbed on finely dispersed dust particles and their spread is larger than that of big dust particles.

In the old Moscow habitation districts, soil pollution with BP is higher than in the new ones (32), which shows the stability of this compound and the possibility of its accumulation.

Of even greater interest are the data obtained by us in the vicinity of large factories which discharge into the air considerable amounts of carcinogenic hydrocarbons. BP concentration in soil samples taken from the territory of an oil refinery reaches 200 mg/kg, which is 400 times as much as its concentration in soil samples collected in the hospital yard of one of the old Moscow districts. Meanwhile the soil around an asphalt plant (in which the bitumens used are free of BP, and where the technological processes provide no conditions for the production of BP) contains almost the same amount of BP as that in other Moscow old residential areas, e.g. an average of 346.6 μg per kg.

In Birulevo (a Moscow suburb) where the main and probably the only source of air pollution with carcinogenic hydrocarbons is the railway station, soil pollution amounts to 75 μg per kg on the average. Approximately the same concentration of BP was found in a field near Moscow located very close to heavy traffic.

Thus, in cities there may exist a definite average level, a “background” of soil pollution with BP and, probably, special sites where the level of BP concentration is particularly high. Soil pollution depends on the overall pollution of the human environment with carcinogenic hydrocarbons and may serve as an indicator of the presence and the level of BP pollution. According to the content of carcinogenic compounds in soil we can
The Role of Microorganisms in the Fate of Benz(a)pyrene

The Role of Microorganisms in the Fate of Benz(a)pyrene in the Soil

Simultaneously, a question arises concerning the fate of hydrocarbons in soil. Recently, in collaboration with Professor M. N. Meissel, Corresponding Member of the U.S.S.R. Academy of Sciences, and his assistants (the Institute of Molecular Biology of the Academy of Science of the U.S.S.R.) we have obtained some data concerning the problem of absorption and transformation of BP by different soil microorganisms.

Some years ago Meissel (16) and A Graffi (Z. Krebsforsch., 52: 234, 1941) independently found that carcinogenic aromatic hydrocarbons can penetrate into microorganism cells and be accumulated there. Both authors used the method of luminescent microscopy. Later on, by means of luminescent microscopic investigations it was shown that BP is bound in cells by cytoplasmic lipoprotein components and free lipid inclusions, is accumulated in cells, and can remain there for a long time or pass to other cells (18). It was found that the yeasts Endomycetes magnusi and Candida lipolytica were capable of absorbing BP contained in nutrient medium. Spectrofluorescent methods showed BP reduction with culture development, and yeast cells remain longer in the medium with BP. This is accompanied by green luminescence, typical of BP metabolites, appearing in animal tissues as a result of oxidation and hydroxylation of this hydrocarbon (19).

The next work (20) deals with cultures of 17 strains of various soil bacteria isolated from the soil taken from territory of an oil refinery and containing BP in the amount of 100,000 µg per kg. All these cultures more or less actively absorbed BP, and accumulated it in their cytoplasm, lipid inclusions, and, in the so-called mesosomes representing analogs of mitochondria. The selected strains of soil bacteria were cultivated in agar nutrient medium, with BP introduced in the concentration of 10 µg per ml. The amount of BP destroyed or modified by microorganisms (i.e. transformed into derivatives having no BP luminescence) was shown by the difference between the amount of hydrocarbon introduced into nutrient medium and the portion of BP remaining in the medium and microorganisms after they had been cultivated for two, three, or four days. The results obtained are presented in Table 2.

As can be seen from Table 2, in two cultures the whole amount of BP remained unchanged while in the other two it gradually disappeared. Special tests with hydrolysis and re-extraction of the biomass showed that disappearance of BP from the nutrient medium and the microbes did not account for its possible binding with proteins and, consequently, was the result of its change.

Thus, it must be recognized that in soil contaminated with BP microorganisms are present which can both accumulate BP (which is typical of all soil bacteria cultures investigated) and also modify it considerably. These species of bacteria probably transform BP into less carcinogenic oxidation products. The example of the most active culture in this respect is N 13, while N 2/II does not fully possess this property.

Results obtained on bacterial cultures N 5 and N 2/II (see Table 2), as well as on cultures NN 1,3,4 present a kind of natural control, as they show that in microorganisms which accumulate BP from the medium but do not transform it during their life, the amount of BP extracted after cultivation totals 100% of that introduced into nutrient medium.

The cultivation of the soil bacteria strains was continued in agar medium free of BP and their capacity to destroy BP was tested. As seen from Table 3, cultivation in medium free of BP results in decrease of this capacity, while addition of BP to the agar medium for the period of 2 months restored this property.

As the capacity of some soil bacteria to destroy BP was confirmed in agar medium, we initiated experiments with original soil. The soil was taken from the territory of the same oil refinery where the bacteria were originally isolated. To a sample of sterilized soil, weighing 10 gm and containing about 200 µg of BP, were added 10 ml of a suspension of soil bacteria in meal-peptone medium. The results are summarized in Table 4. Previous luminescent microscopic investigations showed that the bacteria of 3 strains multiplied in this soil medium intensively, absorbed BP, and did not form spores within 8 days. BP estimation in the sample of sterilized original soil served as control.

<table>
<thead>
<tr>
<th>Test NN</th>
<th>Amount of benz(a)pyrene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extracted (µg)</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Culture N:</td>
<td></td>
</tr>
<tr>
<td>N 13</td>
<td>176</td>
</tr>
<tr>
<td>N 2/II</td>
<td>176</td>
</tr>
<tr>
<td>N 5</td>
<td>201</td>
</tr>
<tr>
<td>N 2/I</td>
<td>198</td>
</tr>
<tr>
<td>Control</td>
<td>208</td>
</tr>
</tbody>
</table>

TABLE 2
The Change in the Total Content of Benz(a)pyrene in the Medium and in the Cells of Soil Microorganisms

<table>
<thead>
<tr>
<th>Period of cultivation</th>
<th>Amount of benz(a)pyrene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extracted (µg)</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>2 days</td>
<td></td>
</tr>
<tr>
<td>3 days</td>
<td></td>
</tr>
<tr>
<td>4 days</td>
<td></td>
</tr>
</tbody>
</table>

JUNE 1967 1135
TABLE 3
Gradual Reduction and Reestablishment of the Capacity to Destroy BP* in Long-Time Cultivation of Soil Bacteria in a Medium Free of BP

<table>
<thead>
<tr>
<th>Date of inoculation of the strain N 13</th>
<th>Amount of BP destroyed during 4 days of cultivation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/24/66</td>
<td>72</td>
</tr>
<tr>
<td>3/3/66</td>
<td>58</td>
</tr>
<tr>
<td>3/13/66</td>
<td>42</td>
</tr>
<tr>
<td>7/3/66</td>
<td>26</td>
</tr>
</tbody>
</table>

* BP, benz(a)pyrene.

** This substrain was cultivated during the last 2 months (May-June) in a medium with BP.

* BP, benz(a)pyrene.

At the end of the cultivation period the strain N 5, which did not destroy BP in an artificial meal-peptone-agar medium was quite active (> 50%) when returned to its “native soil”. The data showed that the soil bacteria can change the carcinogenic hydrocarbon BP in the soil, converting it probably to a less carcinogenic oxidized compound and perhaps even destroying it.

The possibility of the oxidation of BP and other carcinogenic hydrocarbons has been well known for some time. In our experiments BP and DMBA-acetone solutions (concentration 10^-6 gm per ml) were treated by an ozone-air mixture and were destroyed completely within 2.5 minutes for BP and only 1 minute for DMBA (11).

CONCLUSIONS

The results of our work showed the possibility of the quantitative determination of carcinogenic hydrocarbons at different stages of their distribution and circulation both in animal organism and in human environment. In the environment of man we can see a peculiar migration, a kind of circulation of these substances, e.g., their transfer from one product into another, from one sphere of industry into another one, from industrial and transport exhausts into the atmospheric air, etc. Being exhausted into the air by factories, heating systems, and combustion engines, they fall on soil, appear in water, may accumulate or disappear, pass from nutrient medium into microorganisms, and contaminate fodder, and in some cases even the food consumed by man.

Not all of the possible pathways of the circulation carcinogenic hydrocarbons in the human environment have been studied so far, but the data obtained by us concerning the possible transformation of BP by some soil bacteria not only throws light upon the fate of this substance in soil, but makes it possible to conduct biologic purification of the human environment from carcinogenic hydrocarbons similar to that for phenol in sewage which has become the practice of some industrial enterprises. Thus, modern accurate methods of carcinogenic hydrocarbon determination show new ways for cancer prevention.

REFERENCES

7. Danil'tseva-Fedoseeva, G., and Khesina, A. J. The Quasi-
Carcinogenic Hydrocarbons in Human Carcinogenesis


L. M. Shabad


Access the most recent version of this article at: [http://cancerres.aacrjournals.org/content/27/6_Part_1/1132](http://cancerres.aacrjournals.org/content/27/6_Part_1/1132)