On the Fate of Carcinogenic Hydrocarbons in the Animal Body

L. Th. Larionow, M.D.

(From the Department of Pathological Physiology of the Byelorussian State Medical Institute, Minsk, B. S. S. R.)

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The study of the fate of carcinogenic substances in the body was first taken up in 1934-36 by Chalmers and Peacock (8, 17), Berenblum and Kendall (1, 2), Hieger (11), Lorenz and Shear (14) and others. Extension of knowledge in this field is necessary for further progress in studying the mode of action of carcinogenic substances and the pathogenesis of malignant tumors induced by these chemical factors. Moreover, if the carcinogenic hydrocarbons do not greatly differ in their structure and physicochemical properties from the supposed endogenous carcinogenic substances, the study of these questions may yield material for drawing certain analogies with respect to the latter. In this connection it is of interest to study the fate of carcinogenic substances not only in a healthy organism, but also in one affected with pathologic lesions.

The investigations we started in 1940 on some of these problems together with my assistants B. Vigidorovich, I. Plindov, M. Zalesskaya and A. Friedman were interrupted by the war in June 1941 and the spectrograph we had been using was entirely ruined. Data which we had obtained before this interruption are presented in this paper.

MATERIALS AND METHOD

The investigations were carried out on over 100 mice, 60 rats, 50 rabbits, 15 dogs and 1 cat. The carcinogenic hydrocarbons used were for the most part 3,4-benzpyrene, to a lesser extent 20-methylcholanthrene and 1,2,5,6-dibenzanthracene. To get a general notion of the hydrocarbon present in bile, urine, cerebrospinal fluid or at the site of subcutaneous injection visual examination of the fluorescence of these materials in a filtered ultraviolet beam was made. To discover the presence of hydrocarbons in the viscera, in blood and other fluids we usually prepared benzene extracts according to Berenblum and Kendall's second method (2). The fluorescence spectrum of these extracts, reduced to the same volume, was photographed by means of the spectrograph. Most of the investigations were made with a spectrograph manufactured by the Physical Institute of the Leningrad University. It has optical parts made of glass and a dispersion of from 50 Å to 1 mm. in the violet region. Occasionally for the sake of approximate quantitative comparison fluorescence spectra of standard hydrocarbon solutions in benzene were photographed on the same plate. The arrangement of the bands in the spectrum of a benzpyrene solution in benzene at a concentration of 0.3 to 1 μ in 1 cc. as obtained under our conditions of photographing is shown in Table I.

| Table I: Fluorescence Spectrum of Benzpyrene Solution in Benzene at Concentration of 0.5 to 1 μ in 1 cc. |
|----------------------------------|---------------|---------------|---------------|---------------|---------------|
| Band I                          | Band II       | Band III      | Band IV       | Band V        |
| 4,050-4,080                      | 4,100-4,120   | 4,170-4,190   | 4,250-4,430   | 4,520-4,680   |
| 4,580                            |              |              |               |               |

The width of the bands, particularly the position of their long-wave edge is somewhat dependent on both concentration and exposure. The short-wave edge of the bands is more definite. Band IV is the most intense, band I ranks second, then come bands V and II, while band III takes the last place. At the lowest concentrations only I and IV prove to be different (Fig. 1).

THE DISTRIBUTION OF BENZPYRENE IN THE BODY WHEN INTRODUCED INTO THE BLOOD OR SUBCUTANEOUSLY

Hydrocarbon was introduced into the blood in a...

1 Investigations carried out by Assistant Physician I. Plindov.
fine aqueous suspension prepared by precipitation from acetone according to Boyland’s method (4). By means of careful evaporation the suspension was reduced to the desired concentration. One of the series of experiments carried out on mice is further described.

Each of 20 mice was given 1 cc. of an aqueous suspension of 0.3 mgm. benzpyrene administered into the caudal vein. The mice were killed in pairs after different intervals. The blood of every pair was then collected, as well as their lungs, liver, kidneys, spleen, the adipose tissue from the peritoneal cavity (chiefly that surrounding the testes), and the small and large intestine together with its contents. 0.5 of each organ was used for preparing the extracts which were then reduced to an equal volume of 2 cc. and their fluorescence spectra photographed. The data obtained are shown in Table II (the plus signs designate the relative intensity of the bands in the spectrum).

**Table II: The Distribution of Benzpyrene in the Blood and Organs after Injections**

<table>
<thead>
<tr>
<th>Time</th>
<th>Blood</th>
<th>Lung</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Adipose tissue</th>
<th>Upper segment of the small intestine</th>
<th>Lower segment of the small intestine</th>
<th>The large intestine</th>
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<tr>
<td>15 minutes</td>
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<td>+++</td>
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<tr>
<td>6 hours</td>
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Table II shows that within the first hour following the administration of benzpyrene its presence is revealed in varying quantities in all the organs and tissues examined. The lungs and the liver contain larger quantities of benzpyrene than any of the other organs (Fig. 2). The accumulation of benzpyrene in the lungs is accounted for by the fact that its larger particles may block the lung capillaries immediately after the suspension has been introduced into the vein, as was first noted by Peacock (18). On the other hand the concentration of hydrocarbon in the liver is evidently the result of the liver cells retaining the benzpyrene which has been dissolved in blood. Peacock's investigations (17) have made it generally known that the liver eliminates hydrocarbons together with bile. Later we shall return to the discussion of this phenomenon. As long as hydrocarbon circulates in the blood it is also found in the kidneys and the spleen in a concentration almost equal to that of the blood. An undoubted accumulation of benzpyrene takes place in the adipose tissue, which moreover retains it for a longer period of time (24 hours).²

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As a result of benzpyrene being taken up by the viscera, eliminated with urine, as well as with uterus (see below) and possibly partially destroyed, the concentration of hydrocarbon in blood is relatively low and in about 2 to 3 hours benzpyrene is found to disappear from the blood entirely.

Similar results have been obtained on rats. The administration of 1 mgm. of benzpyrene into the blood was followed by its rapid disappearance from the latter (Fig. 3). Within the first hour large quantities of benzpyrene were found in the lungs and the liver,² smaller quantities in the kidneys and the spleen (Fig. 4), but in a short time (in 3 hours) it was no longer found in any of these organs. Hydrocarbon was found to be retained longest by adipose tissue (Fig. 5).

Several experiments carried out on rabbits revealed similar data. When 1 or 2 mgm. benzpyrene are given, the latter appears in the blood within the first 2 to 3 hours following its administration, and not only in whole blood, but in serum as well. Two experiments in which hydrocarbon was added to stabilized rabbit blood in vitro and the plasma and washed erythrocytes extracted separately have shown the coefficient of its distribution between the erythrocytes and the plasma to be practically equal to 1.

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With respect to the intestine it can be seen from Table II (experiments on mice) that hydrocarbon is at first found to be present in all its segments

² The solution of benzpyrene in body fat was first described by Chalmers and Peacock (8).

³ In one of the experiments in which 1 cc. of blood contained about 0.5 mg benzpyrene the extract of 1 gm. of liver was found to contain a quantity of about 5%.
The fluorescence spectra of the extracts drawn from the upper segment of the small intestine in mice proved to be different from those of the other organs: a wider and more intense band (from 4,150 to 4,210 Å) was observed in place of the usually faint band III, while between bands IV and V a new diffuse band of about 4,420 to 4,500 Å in width was found to appear. This latter band seemed to be less distinctly outlined and owing to its close proximity to band V of benzyrene is almost fused with the latter (Fig. 6). It is evident that in this case an overlapping of two different substances—i.e. of benzyrene and of its derivative—has taken place. The position of the latter’s bands approximately coincides with that of the benzyrene derivative which is eliminated with bile (see also below), and obviously enters the intestine together with it. The fluorescence spectrum of the

DESCRIPTION OF FIGURES 1 TO 15

Fig. 1.—Fluorescence spectra of benzyrene solutions in benzene in decreasing concentration: 1 = control benzpyrene spectrum, 2 = spectrum of an extract of rabbit urine collected in 2 hours following an intravenous injection of 1 mgm. benzpyrene.

Fig. 10a.—1 = control benzpyrene spectrum, 2 = spectrum of an extract of rabbit urine collected in 2 hours following an intravenous injection of 1 mgm. benzpyrene.

Fig. 10b.—Analogous experiment on another rabbit: 1 = control benzpyrene spectrum, 2 = rabbit urine previous to benzyrene injection, 3 = in 1 hour following the injection, 4 = in 4 hours, 5 = in 6 hours.

Fig. 10c.—Experiment on mice (0.3 mgm. benzyrene administered into the blood): 1 = control benzpyrene spectrum, 2 = urine in 3 hours' time after injection, 3 = urine in 12 hours, 4 = urine in 24 hours.

Fig. 11.—Fluorescence spectrum of whole bile in dog after an intravenous injection of 2 mgm. of benzyrene: 1 = control benzpyrene spectrum (benzene solution), 2 = bile previous to benzyrene injection, 3 = the same in 30 minutes' time after the injection, 4 = in 1 hour, 5 = in 2 hours, 6 = in 3 hours.

Fig. 12.—Experiments on dog No. 3. May 26, 1941. Phosphorus injected on May 22, 24 and 25, 1941. 1 = control benzpyrene spectrum, 2 = fluorescence spectrum of whole bile in 30 minutes after an intravenous injection of 2 mgm. of benzyrene, 3 = the same in 1 hour, 4 = in 2 hours, and then successively in 1 ½ hours, in 3 hours, in 3½ hours, in 5 hours, in 5½ hours, in 6 hours, in 6½ hours, in 7 hours and in 7½ hours.

Fig. 13.—Extract of cholesterol deposit in a control rat, 2 = extract of cholesterol deposit in 3 hours after an intravenous injection of 1 mgm. benzyrene, 3 = in 9 hours, 4 = in 20 hours, 5 = standard solution of benzyrene in benzene (0.1γ in 1 cc.).

Fig. 14.—Fluorescence spectra of extracts from the stomach of rats (together with the gastric contents) after the administration of 1 mgm. benzyrene in food: 1 = in 3 hours, 2 = in 6 hours, 3 = in 12 hours, 4 = in 24 hours.

Fig. 15.—In 6 hours after the administration of 0.2 mgm. benzyrene in food to a mouse: 1 = extract of the stomach, 2 = extract of the upper and the lower portion of the small intestine respectively, 4 = extract of the large intestine.
benzpyrene derivative eliminated by the liver and
known as BPX\(^4\) was first described by Chalmers.
It is characterized by two wide bands. According
to Chalmers \(5\) BPX, in an alcohol extract of the
bile of mice, has bands at 4,100 to 4,250 and
(4,350 to 4,500).\(^5\)

Benzene extracts obtained either from the lower
segment of the small intestine or from the large
intestine usually were found to contain unchanged
benzpyrene. Only in two of the cases BPX was dis-
covered in these parts of the intestine: in 30
minutes (in the lower part of the small intestine)
and in 24 hours (the contents of the large intestine)
following an injection. In the case of rats no deri-
ivative, similar to the BPX observed in mice, was
ever found to be present in the intestinal contents.

The presence of fairly large quantities of un-
changed benzpyrene in extracts drawn from the
intestine leads to the supposition that hydrocarbon
may be retained from the blood by the intestinal
walls.

With the view of settling this problem additional
experiments were carried out. The contents of the
intestine and its wall washed of the contents were
extracted separately. In parallel experiments made
on rats the vascular system of the animals was previ-
ously washed with normal saline solution by means
of a cannula inserted into the heart. The experi-
ments have shown that the intestinal wall actually
retained benzpyrene. In the case of rats, in particu-
lar, the quantity of benzpyrene present in the wall
of both the small and the large intestine an hour
after its administration proved to be fairly consider-
able, while the intestinal contents had mere traces
of benzpyrene, if any (Fig. 7).

In addition, an experiment was carried out on a
rat in which the lower part of the duodenum was previ-
ously ligated. After the suturing of the ab-
dominal cavity the animal was given the usual dose
(1 mgm.) of benzpyrene intravenously. In 3 hours'
time hydrocarbon was found to be present in the
wall of all the segments of the intestine (the
vascular system had been thoroughly washed), while
the intestinal contents had only barely noticeable
traces (Fig. 8). (The latter phenomenon may be
accounted for either by a partial peeling off of the
mucosa in removing the intestinal contents or by a
partial excretion of hydrocarbon by the intestinal
glands into the lumen of the intestine). In addi-
tion the retention of benzpyrene by the intestinal
wall is confirmed by an experiment the author
carried out together with N. N. Blokhin on a dog
in which vascular cannulae were inserted (after
London's method) into the portal vein and into
one of the hepatic veins. In this case arterial blood
was found to have a fairly high concentration of
hydrocarbon 1 hour and 40 minutes after an in-
travenous injection of 5 mgm. of benzpyrene, while
the blood of the portal vein contained but traces
of it.

**EXPERIMENTS ON SUBCUTANEOUS INJECTIONS OF
BENZPYRENE**

The investigations carried out by my collaborators
Feldman and Furman \(10\) in 1940 helped to prove
that if injected subcutaneously in a solution of
slowly absorbable oil (olive oil) in a dose of 1 mgm.
benzpyrene can be revealed at the site of injec-
tion for as long as 3 months. If injected in a fine
aerosol suspension hydrocarbon is more rapidly
resorbed (injected in a dose of 1 mgm. it was
found to disappear in our experiments in a month's
time; injected in a dose of 0.5 mgm. it disappeared
in 20 days' time).

A series of experiments was carried out on 14
mice which were given injections of 0.2 and 1 mgm.
benzpyrene in an aqueous suspension. Benzene
extracts of the blood contained no hydrocarbon.
Apparently, being slowly absorbed from the sub-
cuts into the blood, benzpyrene is so intensely
withdrawn from it by the viscera that it fails to
be determined in blood by the methods we have
used. The liver, when examined within the first
48 hours following the injection, occasionally con-
tained traces of benzpyrene. In this case the fluo-
rescence of the gall bladder was observed. Within
24 hours after the injection traces of benzpyrene
were also found in the lungs (possibly due to the
retention of dissolved benzpyrene). Traces or even
more noticeable quantities of benzpyrene were also
determined in extracts of the large intestine. Begin-
ning with 6 hours after the injection benzpyrene
was invariably present in the adipose tissue of the
peritoneal cavity (in one of the experiments its
quantity amounted to 0.2\(\gamma\) to 0.3 gm. of adipose
tissue) (Fig. 9).

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\(^4\) Berenblum (Cancer Research, 3:145. 1943) and his
collaborators have found BPX to be 8-hydroxy-3,4-benz-
pyrene.

\(^5\) In his subsequent reports describing experiments car-
rried out mainly on rats Chalmers \(6\) states that in an
alcohol solution of BPX extracted from the feces BPX
has bands at (4,250 to 4,400) and (4,500 to 4,600) A.
The Elimination of Carcinogenic Hydrocarbons by the Liver

The experiments were made on rabbits and consisted of inserting a glass cannula into the animal's common bile duct, and ligating the gall bladder duct. The bile was collected at definite intervals both before and after injecting carcinogenic substances into the blood.

Fourteen rabbits were given intravenous injections of benzpyrene in doses of 1 to 2 mgm. in an aqueous suspension. The first traces of the fluorescent substances were revealed in those portions of bile which had been collected in 10 to 15 minutes, or sometimes 20 minutes, after the injection of benzpyrene. To judge by the intensity of bile fluorescence the most active excretion of the substance begins in 30 to 40 minutes, rarely in an hour's time after the hydrocarbon injection was given, and lasts for about 30 minutes. After that the intensity of bile fluorescence gradually decreases, and in 2 to 2½ hours, less frequently in 3 to 3½ hours after the beginning of the experiment it ceases entirely.

The benzene extract was prepared as follows: the bile was dried by heating, then reduced to powder, and extracted by a small quantity of benzene at room temperature. The fluorescence spectrum revealed 2 intensely manifest wide bands, one between 4,150 and 4,320 A, the other between 4,400 and 4,600 A (occasionally there was a third band of between 4,700 and 4,800 A). According to Chalmers' data in benzpyrene was found to be discharged with urine in the form of a fluorescent derivative, whose spectrum closely resembled that of the substance discharged with bile.

The Excretion of Benzpyrene by the Kidneys

Six rabbits were given injections of 1 mgm. benzpyrene in an aqueous suspension into the auricular vein. Urine was collected either in metabolism cages or drawn by means of a catheter. Mice were injected with 0.5 mgm. hydrocarbon into the caudal vein. The urine of 5 animals was collected as follows: it was allowed to run through a wire netting in the bottom of the glass jar which retained the feces. A mere visual examination of the urine collected within the first 4 to 6 hours in the case of rabbits and 3 hours in the case of mice showed a violet-blue fluorescence. Benzene extracts revealed a typical fluorescence spectrum with two broad bands of between 4,150 and 4,300 A and 4,420 to 4,600 A (Fig. 10). In case of higher concentration there appeared a third barely noticeable band of between 4,700 to 4,800 A. Thus, benzpyrene was found to be discharged with urine in the form of a fluorescent derivative, whose spectrum closely resemble that of the substance discharged with bile.

Do Carcinogenic Hydrocarbons Penetrate through the Hematoencephalic Barrier?

Among the problems concerning the distribution of carcinogenic substances in the body the possibility of their penetrating into the cerebrospinal fluid is of particular interest.

Accordingly experiments were made on rabbits, dogs and cats with the view to investigating the problem. The method used in the experiments consisted in drawing the cerebrospinal fluid by means of suboccipital punctures at different intervals following an injection of a benzpyrene suspension into the blood. The fluid was first tested visually with respect to its fluorescence, and then the fluorescence spectrum was photographed by means of a spectrograph. In some cases a benzene extract of the fluid was made, which was also examined spectrographically. Ten experiments were carried out on rabbits. The interval of 30 minutes of 1 or 2 injections with an interval of 30 minutes of 1 or 2 mgm. benzpyrene. The fluid was drawn at intervals of from 1 to 3 hours following the injection. Normal cerebrospinal fluid was found to produce a very faint bluish-grey fluorescence. After an injection of benzpyrene a visual test of the fluid revealed that its fluorescence in no way differed from that of the control fluid.

7 Investigations carried out by I. P. Plindov.
8 Investigation carried out by the writer together with M. Zalesskaya.

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6 Investigations carried out by A. Friedman.
spectrographic examination of the fluid or its extract which was made in the case of 3 rabbits, failed to reveal any benzpyrene bands. At the same time extracts of the arterial blood of two rabbits produced a spectrum of benzpyrene fluorescence.

Similar experiments with doses of from 2 to 4 mgm. benzpyrene were also carried out on 3 dogs. The fluid was drawn at intervals of from 1 to 24 hours following the injection of hydrocarbon. A spectrographic study of the fluid did not reveal any benzpyrene bands. When 2 mgm. benzpyrene were injected into a cat no hydrocarbon was discovered in its cerebrospinal fluid after an interval of 3 hours (the blood test was positive).

Thus, these preliminary data have shown that in the larger laboratory animals benzpyrene when injected into the blood, at least when it is given in small doses, is not detected in the cerebrospinal fluid.

In this connection it seemed interesting to study the question of benzpyrene solubility in the cerebrospinal fluid (according to our data 100 cc. of blood plasma dissolves up to 4 to 6 mgm. of benzpyrene). To study the problem we added to 2 cc. of the cerebrospinal fluid of a dog 0.02 mgm. benzpyrene in an aqueous suspension. After allowing the liquid to stand for 3 hours in a thermostat at 37° C. it was thoroughly centrifuged. A visual examination showed a typical blue-violet fluorescence and a spectrographic examination—the usual bands of dissolved benzpyrene (the suspension of undisolved benzpyrene particles produces a greenish-yellow fluorescence and a correspondingly peculiar spectrum). Consequently the solubility of benzpyrene in the cerebrospinal fluid is by no means very low.

Only after these experiments had been carried out we learned about a paper published by Peacock (18) in which he stated that part of the benzpyrene injected into the blood of mice was found to penetrate into the central nervous system. Bergoltz (3) also found a quantity of benzpyrene in the brain of mice in which an intravenous injection of 6 mgm. of hydrocarbon in ol. persicarum was made.

DO CARCINOGENIC HYDROCARBONS PENETRATE INTO THE MILK?  

The question as to whether carcinogenic substances penetrate into the milk of a nursing mother is of great interest from the view point of its possible effect on the offspring, as well as in connection with the well-known fact that the action of tar and carcinogenic hydrocarbons increases the incidence of carcinomas of the mammary gland in mice (Soboleva [19], Larionow [12], Maisin and Coolen [15], and others).

We have carried out 2 experiments, one on a rabbit, the other on a dog. The female rabbit, which had had a miscarriage, was injected in the course of 3 days twice daily with 2 mgm. benzpyrene in fine aqueous solution into the aural vein. Milk was drawn daily from the mammary glands in quantities varying from 1 to 4 cc. at different intervals following the benzpyrene injection. The milk was extracted with benzene according to Berenblum and Kendall's method and the fluorescence spectrum of the extracts was then photographed. Only the spectrum of 1 of the 4 extracts obtained was found to exhibit a barely noticeable IV benzpyrene band, while the rest of the extracts had no benzpyrene bands at all.

The dog under experiment was left only one puppy to suckle. In the course of 3 days it was injected intravenously with 2 to 4 mgm. of benzpyrene in aqueous suspension daily. Milk was drawn every day (sometimes twice daily) for 4 successive days. During the course of benzpyrene injections the total number of milk tests taken was 6 (from 2 to 4 cc. each). Photographs of the fluorescence spectrum of benzene extracts from the milk did not exhibit any benzpyrene bands.

On the ground of these preliminary experiments we are apt to doubt that benzpyrene in an unchanged form was excreted with the milk of rabbits and dogs in any noticeable quantity.  

THE ELIMINATION OF BENZPYRENE BY THE LIVER WHEN IT IS AFFECTED WITH PATHOLOGIC PROCESSES  

A metal fistulous cannula was inserted under aseptic conditions into the gall bladder of dogs and the end of the cannula was made to protrude through an incision of the skin. The common bile duct was ligated in the course of the operation. The incisions having healed, numerous experiments were carried out on the same dog.

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9 Investigation carried out by the writer together with M. Zalenskaya.

10 According to Peacock's (18) data, which became available to us only after we had made these experiments, when intravenous injections of benzpyrene were given to mice an excretion of hydrocarbon into milk was found to occur.

11 Investigations carried out by A. Friedman.
Preliminary experiments carried out on 3 dogs showed that the animals were suitable objects for studying the elimination of carcinogenic hydrocarbons with bile for a period as long as several weeks and that the results they yielded were consistent. When 2 mgm. benzpyrene was injected into the blood, a typical blue-violet fluorescence appeared in portions of bile collected in 20 to 30 minutes following the injections, usually reached its maximum by the end of the first hour and mostly disappeared in 3 1/2 hours (less frequently in 3 or 4 hours).\textsuperscript{12}

Photographs of the fluorescence spectrum of whole bile revealed 2 diffuse bands peculiar to BPX. However, as compared to the benzene extracts of rabbit bile containing BPX (see above), these bands were situated nearer the short-wave part (approximately by 50 Å), and besides, their margins (the short-wave margin of the first band in particular) were far more indistinct (Fig. 11).

Experiments on dogs showed besides that the portions of bile containing the fluorescent benzpyrene derivative rapidly turned green. Whereas normal bile preserved for a long time its olive-yellow color, bile discharging the benzpyrene derivative acquired within the first few hours a bright green, which was the more intense the greater the quantity of fluorescent substances it contained. Apparently, this acquired green color depended upon the oxidation of bilirubin into biliverdin which rapidly developed in the presence of the benzpyrene derivative excreted by the liver.

Experiments on the induction of pathological processes in the liver were carried out on 3 dogs. Hepatitis was brought about in 2 dogs by giving them injections of allylformate in the abdominal cavity. Control experiments carried out on the same dogs before they were injected with allylformate had shown that following an injection of 2 mgm. benzpyrene into the blood the elimination of its derivative by the liver ceased in 3 to 3 1/2 hours' time. Following the first injection of allylformate (0.15 cc. in 200 cc. of normal saline solution) no particular changes in the process of elimination were found to develop. After the second injection which was given 10 days after the first, both dogs soon exhibited a considerably lengthened period of eliminating the benzpyrene derivative with the bile. When 2 mgm. hydrocarbon was injected, the elimination lasted for 6 to 7 hours. The most intense elimination began half an hour later than in control experiments. This lengthening of the elimination period is apparently connected with a decrease in the rate of bile excretion.

Indeed, the total amount of bile discharged during the 5 hour duration of the experiment was reduced to almost half its previous quantity; for instance in dog No. 2 on the average from 20 cc. to 11 cc. Thus, the benzpyrene derivative was eliminated both previous and subsequent to the lesion of the liver by the same quantity of bile but over a longer period of time. Thus, in dog No. 1 benzpyrene was eliminated in control experiments in 14 cc. of bile (in the course of 3 hours), whereas after the second injection of allylformate it was eliminated in 15 cc. (in the course of 6 to 7 hours). No fall in the intensity of bile fluorescence was observed. Apparently, the liver, in spite of its being affected with a pathological process, excreted about the same quantity of the hydrocarbon derivative.

By the end of the experiment the first dog developed fairly marked ascites. It was killed on the 24th day. The second dog died in 10 days following the second injection of allylformate. The autopsy of the first dog showed its liver to be greatly reduced in volume. A microscopic study gave evidence of hepatitis and perihepatitis with prevailing phenomena of atrophy of the liver parenchyma. In the second dog a microscopic examination also revealed hepatitis with phenomena of initial cirrhosis.

In the third dog the lesion of the liver (fatty degeneration) was induced by means of phosphorus (three subcutaneous injections of 0.4 cc. 1 per cent ol. phosphorati). In this dog as well, the duration of benzpyrene elimination was increased from 3 1/2 hours first to 7 and then to 12 hours. The beginning of intense elimination was delayed by 30 minutes. Contrary to the experiments with allylformate, poisoning with phosphorus did not reduce the quantity of bile excreted in a time unit. Consequently, the fluorescent substance was discharged in far greater quantities of bile as compared with controls (on the average it was 12 cc. previous to the injection of phosphorus and 20 to 30 cc. following it). The extent of bile fluorescence was fairly marked, as shown both by a visual test and by spectrographic examination (Fig. 12). The impression was that fatty degeneration of the liver caused a larger quantity of fluorescent substances to be eliminated with the bile than that eliminated by a healthy liver. This was possibly due to a larger quantity of benzpyrene having been dissolved in the fats and lipoids of the liver. The appearance of the fluorescence spectrum of whole bile in no way differed from that of controls (Fig. 12). The dog

\footnote{Similar data were obtained in the case of injections of 5 mgm. methylcholanthrene.}
died in a fortnight following the last injection of phosphorus. Microscopic examination revealed moderate diffuse fatty degeneration of the hepatic cells, characterized by the fatty droplets being very small in size.

The possibility of the benzpyrene, contained in the blood, penetrating into the local lipid accumulations was investigated in the following experiment. Four rats were injected subcutaneously with 10 mgm. cholesterol in a fine aqueous suspension. In 3 weeks the rats were injected intravenously with 1 mgm. benzpyrene. Then one by one the rats were killed in 3, 6, 9 and 20 hours after the latter injection and benzene extracts were drawn from their cholesterol granulomas. The first 3 extracts were found to contain about 0.2y benzpyrene (Fig. 13). Identical experiments on mice yielded similar results. In one of our experiments on rabbits which were injected intravaneously with benzpyrene, it was found to be contained in their suprarenal glands, apparently in the lipoids of the cortical substance.

**Administration of Carcinogenic Hydrocarbons through the Gastrointestinal Tract**

Experiments were made on rats and on mice. In one of the experimental series hydrocarbon (3,4-benzpyrene or 20-methylcholanthrene) was dissolved in sunflower-seed oil, in the other series it was dissolved in milk. In the latter case an aqueous suspension of hydrocarbon was added to the milk from 12 to 20 hours previous to administration. Before the experiment the animals were kept without food for about 12 hours. After that they readily drank several cc. of milk containing the hydrocarbon or ate bits of toasted bread (1 gm.) soaked in several drops of an oil solution of hydrocarbon. The animals were killed at different intervals after the intake of food, and their gastrointestinal tracts were then examined first visually in a filtered ultraviolet beam and then their benzene extracts were subjected to spectrography.

An examination of the stomach of mice and rats fed with hydrocarbon solution besides revealing the fluorescence of the gastric contents, also showed within the first hours, an intense fluorescence of the forestomach mucosa even after it had been thoroughly washed of contents. The mucosa of the glandular portion of the stomach produced no fluorescence. Evidently hydrocarbon was prevented from penetrating into this part of the gastric wall by the mucous secretion with which it is coated. The forestomach continued to be fluorescent even when the gastric contents had ceased to produce any fluorescence. In additional experiments the phenomenon was observed as late as 48 hours following the intake of food. The capacity of forestomach mucosa to absorb benzpyrene and retain it for a considerable length of time—a capacity lacking in the mucosa of the glandular stomach—should be attributed to the structure and chemical composition of its epithelial coat (stratified squamous epithelium). This peculiar property fully explains why any attempt to induce tumors of the stomach by adding carcinogenic hydrocarbons to food resulted only in bringing about tumors of the forestomach but never of the glandular portion of the stomach (13, 16, 20).

In experiments carried out on mice, fluorescence of the gall bladder was often observed within the first few hours, which seems to indicate that benzpyrene absorbed into the blood for the most part in the stomach was eliminated by the liver.

The results obtained by spectographic examinations of benzene extracts from different portions of the gastrointestinal tract (together with their contents) performed at different intervals following the oral administration of 0.5 mgm. benzpyrene in oil solution to rats are shown in Table III (the number of plus signs corresponds to the intensity of the bands in the spectrum).

**Table III: Distribution of Benzpyrene in Segments of the Gastrointestinal Tract of Rats after Oral Administration**

<table>
<thead>
<tr>
<th>Time</th>
<th>Stomach</th>
<th>Upper segment of the small intestine</th>
<th>Lower segment of the small intestine</th>
<th>The large intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 hours</td>
<td>++++</td>
<td>—</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>6 &quot;</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>12 &quot;</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>24 &quot;</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Table III shows the gradual migration of unchanged hydrocarbon into the large intestine. The presence of small quantities of benzpyrene in extracts from the lower segments of the small intestine as early as 3 hours, in spite of its being absent in the upper segment, should be attributed to the fact that the benzpyrene which was absorbed by the blood and included in the general circulation was retained by the intestinal wall (see above). The lasting presence of hydrocarbon in an extract from the stomach is due to its having been retained by the forestomach wall (Fig. 14). The results ob-

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13 Investigation carried out by B. I. Vigdorovich.
14 The phenomenon has been described in a paper published by our collaborators Feldman and Furman.
tained in analogous experiments on mice which were given 0.2 mgm. benzpyrene are shown in Table IV.

TABLE IV: DISTRIBUTION OF BENZPYRENE IN SEGMENTS OF THE GASTROINTESTINAL TRACT OF MICE AFTER ORAL ADMINISTRATION

<table>
<thead>
<tr>
<th>Time</th>
<th>Stomach</th>
<th>Upper segment of the small intestine</th>
<th>Lower segment of the small intestine</th>
<th>The large intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 hours</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>6 &quot;</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12 &quot;</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>24 &quot;</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

The fluorescence spectrum invariably revealed bands of unchanged benzpyrene (Fig. 15).

Benzpyrene taken in milk produced on the whole similar results. The liver extract in these experiments produced the fluorescence spectrum of benzpyrene as early as 3 hours after food was given. Identical experiments were made with methylcholanthrene and yielded similar results. As early as 3 hours methylcholanthrene was found to be present in the extract from the large intestine, where it apparently was first carried by blood circulation. The greater part of the hydrocarbon, however, was only gradually transferred in the course of 24 hours into the lower portions of the intestine and was excreted with the feces. Part of the methylcholanthrene was retained by the wall of the forestomach. Extracts of the intestine, examined spectrophotographically, produced the fluorescence spectrum of unchanged-methylcholanthrene (it was also present in the feces).

SUMMARY AND CONCLUSIONS

The distribution of 3,4-benzpyrene within the animal body was studied on mice and rats which were given intravenous or subcutaneous injections of benzpyrene in a fine aqueous suspension. For this purpose benzene extracts were made from the blood, the liver, the lungs, the spleen, the kidneys, the intestine and the adipose tissue. The fluorescence spectra of the extracts were photographed by means of a spectrophotograph.

The spectrophotographic method was likewise used for studying the elimination of benzpyrene derivatives with bile in rabbits and dogs, and its excretion with urine in mice and rabbits. The elimination of benzpyrene derivatives by the liver was also studied in dogs in which pathologic lesions of the liver (hepatitis, fatty degeneration) had been experimentally induced.

The possibility of benzpyrene penetrating through the hematoencephalic barrier was studied by examining the cerebrospinal fluid drawn from rabbits and dogs. Experiments were also carried out with the view of determining whether or not benzpyrene penetrated into the milk of a dog or a rabbit suckling its young. Finally, the fate of carcinogenic hydrocarbons administered into the gastrointestinal tract was also studied. The investigations were started in 1940 and interrupted by the war in 1941.

The following conclusions may be drawn:

1. If 3,4 benzpyrene in aqueous suspension is injected intravenously its larger particles block the capillaries of the lungs. The smaller particles which circulate in the blood are dissolved by it in a fairly short time (later the particles retained in the lungs are also dissolved). Judging by preliminary data the hydrocarbon is more or less equally distributed between the erythrocytes and the plasma. In a short time, however, hydrocarbon disappears from the blood.

2. Benzpyrene is taken up from the blood by the liver, where it is found to be present (in an unchanged state) in a far higher concentration and from where it is eliminated together with bile into the intestine, as a fluorescent derivative having a peculiar fluorescence spectrum. 20-Methylcholanthrene and 1,2,5,6-dibenzanthracene are also eliminated by the liver.

3. Some of the benzpyrene is excreted by the kidneys together with urine, as a fluorescent derivative having the same fluorescence spectrum as the substance excreted with bile.

4. Part of the benzpyrene circulating in the blood is retained by the intestinal wall.

5. A considerable part of the benzpyrene is dissolved in the body fat and is retained by it for a considerable length of time, thus forming a kind of depot in the adipose cellular tissue.

6. When introduced subcutaneously benzpyrene is also found to be present in the body fat as well as in the liver, the intestine and the lungs (in small quantity).

7. If small quantities of benzpyrene are injected into the blood it is not found in the cerebrospinal fluid of rabbits and dogs.

8. To judge by preliminary data no noticeable quantity of benzpyrene in the form of fluorescent substances has even been observed to be excreted together with milk by rabbits or dogs.

9. In experimental hepatitis in dogs the benzpyrene injected into the blood is eliminated by the liver for a longer period of time because of the reduced rate of bile excretion.
10. In experimental fatty degeneration of the liver, the length of the elimination period is also increased, as well as the quantity of the benzpyrene derivatives which is excreted by the liver.

11. When benzpyrene or methylcholanthrene, administered in fat or oil solution, are added to food a considerable part of the hydrocarbons does not undergo any changes in the intestine and is excreted together with the feces in an unchanged state.

12. The forestomach mucosa in mice and rats is capable of absorbing hydrocarbons and of retaining them for a considerable length of time.

13. Part of the hydrocarbons is absorbed from the gastrointestinal tract (partly even in the stomach) into the blood and can be found, for instance, in the liver.

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L. Th. Larionow


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