Deposition of Benzo[a]pyrene in Mouse Fat after Oral Administration*

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SUMMARY

Deposition of orally administered benzo[a]pyrene (BP) in the adipose tissue (and therefore the mammary gland) of mice depended upon the type of solvent, the concentration of BP in the test meal, and upon the genetic constitution of the test animal; the presence or absence of a gall bladder did not affect the amount of BP taken up by the fat. When the volume of a 1 per cent BP in olive oil meal was varied from 0.05 to 0.4 ml., the concentration of BP in the fat increased only up to a limit reached when 0.15 ml. of solution was fed. Triocanoin was less effective than olive oil as a solvent. In most strains there was no difference between Tween 60 and olive oil, but in C57/St mice Tween 60 was a more effective solvent. The 22 stocks of mice that were studied could be divided into two groups, those giving high levels of BP in the fat and those giving low levels. This grouping followed a pattern consistent with the genetic origin of these stocks. In four strains there was a significant effect of sex on the deposition of BP in the fat. It is suggested that some of the differences among mice of different sexes and strains were due to differences in the body weight.

In rats (9, 16, 17, 23) and mice (1, 2, 14, 19) oral administration of polycyclic hydrocarbon carcinogens is followed by the appearance of mammary carcinomas. Induction of mammary cancer in these cases probably depends upon deposition of carcinogen in the mammary fat pad, and this deposition, in turn, is related to the localization of carcinogen in the general body fat (7, 8). Twenty-four hours after a feeding of carcinogen in sesame oil, the hydrocarbon is found in moderate concentrations in the adipose tissues—including the mammary fat pad—but only in very low concentrations in other tissues (10). 3-Methylcholanthrene (MC) is capable of moving from the fat pad to the glandular cells of the mammary tissue, as is indicated by appearance of carcinogen in milk concurrently with rapid loss of MC from the whole mammary glands of lactating rats (11).

Mammary tumor induction by oral carcinogens depends upon the strain and species of the test animal. Sprague-Dawley rats are very sensitive, developing mammary tumors in 100 per cent of the animals, with a mean induction period of about 65 days after a single feeding of 20 mg. of 7,12-dimethylbenz[a]anthracene (9). Tumors have been produced in other strains of rats (17) and in several strains of mice (1, 2, 14); but relatively greater doses have been used, and long induction periods are required for tumor development. These differences among species and strains may result from different responses of the breast after exposure to equivalent amounts of carcinogen. Variations in endocrine balance might cause such differences (12). Conversely, differences might also result from variations in the amount of carcinogen actually delivered to the mammary gland. The present study was undertaken to evaluate this latter possibility.

MATERIALS AND METHODS

Mice 60–105 days of age were lightly anesthetized with ether and were intubated with a polyethylene tube (outer diameter, 1.3 mm.) attached to a hypodermic needle. Generally, each animal was weighed and then fed 0.2 ml. of 1 per cent benzo[a]pyrene (BP) in olive oil; but in several experiments either the volume, the concentration, or the solvent was changed to provide information relating the experimental method to the results. After 24 hours the mice were killed by cervical dislocation, and a sample of abdominal fat was removed for analysis. The fat was weighed and was analyzed for BP by a method devised to measure hydrocarbons in mouse skin (6). Briefly, the tissue was heated with 1 ml. of aqueous 10 per cent KOH and 1 ml. of 95 per cent ethanol in a 25-ml. Erlenmeyer flask at 60° C. for about 30 minutes. Then 5 ml. of cold water and either 5 or 10 ml. of benzene was added to the saponified tissue. The mixture was shaken thoroughly and was allowed to stand until the layers separated. An aliquot of the benzene extract was removed, treated with a little anhydrous sodium sulfate to remove traces of water, and analyzed in an Aminco-Bowman spectrophotofluor-
ometer. Narrow slits were employed to provide high selectivity, and a standard solution was read before and after each sample to insure stability of instrument response.

During the second half of the experiments every animal was examined under ultraviolet light before removal of the fat. This precaution was undertaken when it was discovered that a newly assigned technician would occasionally force the polyethylene tube through the stomach wall before delivery of the carcinogen. Although the technic was easily corrected, it was considered desirable to check the placement of hydrocarbon routinely. Extra-gastric administration of BP was disclosed by an extremely intense fluorescence of the abdominal fluid, in contrast to a moderate fluorescence in animals properly handled.

In one experiment the cystic duct was tied, and the gall bladder was removed from each of 27 male C57/St mice. One week later, ten of the cholecystectomized mice—those which appeared to have recovered best and were outwardly normal—and ten control animals were fed 0.2 ml of 2 per cent BP in olive oil. The abdominal fat was analyzed for BP content after 24 hours.

RESULTS AND DISCUSSION

The nature of the compound measured.—Several metabolites of BP possess the same ring structure as the parent hydrocarbon and have similar fluorescent properties (15). It was thus necessary to identify the fluorescent compound actually measured by the procedure. After analysis the samples from about 150 mice were pooled, concentrated, and studied by column chromatography with alumina and heptane-benzene mixtures. Only one fraction with BP-like fluorescence could be recovered. This partially purified material was condensed and chromatographed on paper, according to the method of Maly (20). Only one fluorescent spot was observed, either from the sample or from a mixture of sample and reference BP. This evidence indicated that only unchanged BP was measured in the original analyses. No metabolites having BP-like fluorescence had been extracted from the aqueous KOH by the benzene. These results were similar to those obtained in an earlier study of BP in mouse skin (4).

Experimental variability.—The standard errors of the means are presented in the respective tables of this report. The data from these experiments were much more variable than those obtained in studies of BP in mouse skin (3, 6), although the same analytical method was employed for both studies and both covered the same period of time. The greater variability in this paper is due, therefore, to variations in either the treatment or the response of the animals to treatment.

Undoubtedly, some of the variability arose from regurgitation of the administered hydrocarbon. Examination of the cages under ultraviolet light showed that regurgitation did occur at times, but the extent of regurgitation and its contribution to variability were not determined. It might be thought that a major source of experimental variability rested in the difficulties of administration of measured small volumes of a viscous solution through a small bore tube. However, the dose employed in most cases was such that small changes in the volume of administered BP did not affect the results (Table 4).

An additional source of variability was associated with the body weight of the mice. In these experiments, the mice were of approximately the same age, and within the same strain and sex there was little variation in weight. Thus, the effects of body weight were difficult to ascertain. Nevertheless, in eight stocks significant correlation between body weight and concentration of BP in the fat was observed (Table 1). In every case this correlation was negative. In eleven additional stocks the correlation coefficient did not meet the 5 per cent test of significance but did provide a P of 0.2 or less. In nine of these eleven cases the correlation was negative. We concluded that, in heavy animals, the BP was distributed throughout a larger pool of body fat and that the concentration was correspondingly lower. This hypothesis was subsequently confirmed in mice having greatly different body weights (5).

A final source of variability was demonstrated in a few mice having very small stores of body fat. From these animals it was impossible to collect a sample of fat which did not contain excessive amounts of nonadipose connective tissues. Concentrations of BP in the adipose tissue were low because the fat content of the tissue was low, being diluted with large quantities of BP-poor structural material. In most stocks these animals were rejected because they were obvious "runts." However, in young female mice of the I strain, body fat levels were so low...
that satisfactory samples could not be obtained consistently.

The effect of solvent.—Natural oils (sesame oil, olive oil, peanut oil, etc.) often have been used as the solvent for orally administered hydrocarbons. Nevertheless, trioctanoin (tricaprylin), a synthetic saturated triglyceride, had provided certain advantages over natural oils as a solvent for subcutaneous carcinogens (18, 24). That being the case, in our first intubation studies with mice trioctanoin was also used as a vehicle. The results were disappointing, because there was very little carcinogen in the fat, in comparison with results from our earlier studies with rats fed hydrocarbons dissolved in natural oils. We therefore decided to compare trioctanoin with more commonly used oils, feeding 1 per cent BP in 0.1 ml. of oil to C57/St male mice.

The results (Table 2) showed that trioctanoin was much less effective as a vehicle than was either olive oil or sesame oil. Although somewhat higher values were obtained with olive oil than with sesame oil, this difference was not statistically significant. Deposition of BP in fat involved absorption of the hydrocarbon, transport to the adipose tissue, and absorption of the hydrocarbon into the fat cells. Metabolism of hydrocarbon by the liver competed with the process (15). It has been suggested that, to reach the adipose cells in quantity, the hydrocarbon must be absorbed by the lacteals and transported in chylomicrons, thus bypassing the portal circulation (7, 22). In this process, the vehicle is intimately involved. The effectiveness of olive and sesame oils compared with trioctanoin may be due to differences in the digestion and absorption of these natural and synthetic oils. The evidence presented here supports the previous findings that localization in fat of orally administered carcinogens depends upon the vehicle employed. In an earlier experiment, feeding very large amounts of MC in aqueous suspension did not produce high concentrations of the material in rat fat (11).

Twee 60 and related compounds have been used as vehicles for orally administered hydrocarbons, and penetration of BP into the glandular stomach wall is markedly enhanced by some of them (13, 21). In six strains of mice substitution of Tween 60 for olive oil had no significant effect on the localization of hydrocarbon in the body fat (Table 3). On the other hand, in three separate experiments more BP appeared in the fat of C57/St mice when Tween 60 was used. This difference in response among the different strains requires further investigation.

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**Table 2**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Conc. in fat (μg/gm ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olive oil</td>
<td>3.1 ± 0.5</td>
</tr>
<tr>
<td>Sesame oil</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>Trioctanoin</td>
<td>1.3 ± 0.3</td>
</tr>
</tbody>
</table>

* 0.1 ml. of 1 per cent BP fed 24 hours prior to analysis. Ten mice were used in each group.

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**Table 3**

<table>
<thead>
<tr>
<th>STRAIN*</th>
<th>Olive oil</th>
<th>Tween 60</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. mice</td>
<td>Conc. of BP in fat (μg/gm ± S.E.)</td>
</tr>
<tr>
<td>C3H</td>
<td>17</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>CBA</td>
<td>10</td>
<td>6.7 ± 1.5</td>
</tr>
<tr>
<td>CHI</td>
<td>6</td>
<td>10.3 ± 3.7</td>
</tr>
<tr>
<td>JK</td>
<td>7</td>
<td>55.3 ± 11.2</td>
</tr>
<tr>
<td>(A × C3H) F1</td>
<td>9</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>L</td>
<td>8</td>
<td>4.1 ± 0.7</td>
</tr>
<tr>
<td>C57/St</td>
<td>9</td>
<td>3.0 ± 0.5</td>
</tr>
<tr>
<td>C57/St</td>
<td>9</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>C57/St</td>
<td>10</td>
<td>4.6 ± 0.5</td>
</tr>
</tbody>
</table>

* Fed 0.2 ml. 1 per cent BP; sacrificed in 24 hours.
† P < .01.
‡ 0.05 > P > .01.

**Chart 1**

Effect of administered BP concentration on BP concentration in mouse fat. C3H male and female mice were fed 0.2 ml. of BP in olive oil. The lines indicate the 95 per cent confidence intervals.

Effect of dose of hydrocarbon.—When the concentration of BP was varied from 0.5 per cent to 2.0 per cent, the volume being held constant at 0.2 ml., the recovery of hydrocarbon in the fat of C3H mice increased continuously (Chart 1). In contrast, when the volume of solution fed to the mice was varied from 0.05 ml. to 0.4 ml., with the concentration of BP being held constant at 1 per cent, the ultimate concentration of BP in the fat increased only up to the limit reached when 0.15 ml. of solution was fed (Table 4). It appeared that only a limited volume of solution was involved in the process of BP deposition in the fat.

In a further study of the effect of the volume of the test meal, C3H mice were given 0.13–0.40 ml. of olive oil, with concentrations adjusted so that each mouse received 2 mg. of BP. Analysis of the fat after 24 hours showed that deposition of BP in the fat varied markedly, even though the total dose of BP was the same (Table 5). Column 5 shows the calculated volume of administered solution which contained the BP deposited in the adipose tissue. The calculated values are nearly constant, suggesting
Effect of feeding various volumes of 1 per cent BP in olive oil

<table>
<thead>
<tr>
<th>Volume of solution fed* (mL)</th>
<th>Total dose of BP fed (mg.)</th>
<th>No. mice</th>
<th>Conc of BP in fat (µg/gm ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.0</td>
<td>10</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>0.05</td>
<td>0.5</td>
<td>10</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>0.10</td>
<td>1.0</td>
<td>20</td>
<td>2.8 ± 0.1</td>
</tr>
<tr>
<td>0.15</td>
<td>1.5</td>
<td>10</td>
<td>6.2 ± 0.6</td>
</tr>
<tr>
<td>0.20</td>
<td>2.0</td>
<td>10</td>
<td>6.2 ± 0.5</td>
</tr>
<tr>
<td>0.30</td>
<td>3.0</td>
<td>10</td>
<td>6.5 ± 0.4</td>
</tr>
<tr>
<td>0.40</td>
<td>4.0</td>
<td>10</td>
<td>6.5 ± 0.4</td>
</tr>
</tbody>
</table>

* C3H male and female mice, each fed 2 mg. of BP in olive oil 24 hours prior to analysis.

Effect of volume and concentration of test meal on deposition of BP in mouse fat

<table>
<thead>
<tr>
<th>Vol of solution fed* (mL)</th>
<th>Conc of BP in fat* (µg/gm ± S.E.)</th>
<th>No. mice</th>
<th>Conc of BP deposited in fat (µg/gm ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.13</td>
<td>15.0</td>
<td>10</td>
<td>7.2 ± 0.8</td>
</tr>
<tr>
<td>0.20</td>
<td>10.0</td>
<td>10</td>
<td>4.9 ± 0.4</td>
</tr>
<tr>
<td>0.30</td>
<td>6.7</td>
<td>10</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>0.40</td>
<td>5.0</td>
<td>9</td>
<td>2.8 ± 0.3</td>
</tr>
</tbody>
</table>

* C3H male and female mice, each fed 2 mg. of BP in olive oil 24 hours prior to analysis.

Effect of the gall bladder.—Cholecystectomized mice did not differ from normal animals in respect to the localization of BP in the fat (Table 6). This experiment was prompted by the observation, at the time of the experiment, that less hydrocarbon was found after feeding in mouse fat than in rat fat. From several strains of mice fed about 60 µg of BP/gm body weight, about 3 µg/gm fat was recovered, whereas in Sprague-Dawley rats, feeding only 40 µg/gm body weight produced about 9 µg hydrocarbon per gram of fat. Similar high fat levels were obtained in Fisher rats. Because of the role of bile in lipid digestion, it seemed possible that absorption of the hydrocarbon would be affected by the gall bladder, which is present in mice but absent in rats. The experiment showed, however, that the differences in BP deposition did not depend upon the presence or absence of the gall bladder.

Effect of strain of mouse.—The extent of deposition of BP in the fat of mice of various strains is presented in Table 7. The data reveal that the mice form two major groups, those with BP concentrations below 8 µg/gm of fat and those with BP concentrations above 18 µg/gm.

It is of interest to compare these results with those in Chart 2, which shows the common ancestry of some of these mouse strains. Closely related strains localized BP to a similar extent. Thus, the “C family” (C, CBA, C3H, and DBA/2) all gave high values. A, BRSUNT PB, H, and LOW all gave high values as did the I and JK stocks from which they were derived. Evaluation of the genetics of BP localization in mouse body fat is under way.

Females generally had higher levels of BP in the fat, but in most strains the sex difference was not statistically significant. In four strains the difference between females and males was significant (Table 7). Preliminary studies to be reported later suggest that this difference is secondary to the relationship between body weight and sex. It has
been pointed out earlier in this paper that heavier mice have less BP in the fat than lighter mice. Small variations in BP content of fat among mouse stocks may result from differences in body fat among the strains. Such a relationship would result from solution of a constant amount of hydrocarbon in a greater or lesser amount of fat.

Use of orally administered hydrocarbons to study carcinogenesis.—The data presented here demonstrate that deposition of BP in the general body fat of mice depends on the nature of the solvent, the concentration of the hydrocarbon in the test meal, and the genetic composition of the animal. Evidence previously obtained in rats showed that the molecular structure of the hydrocarbon was of less importance (7). Therefore, the data obtained with BP may be extrapolated to cover other carcinogens.

Oral administration of hydrocarbons provides a convenient method to apply carcinogens to a target tissue without local trauma. Deposition of carcinogen within the target area is a result of physiological processes which are relatively constant, even in extremes of endocrine imbalance (7). Huggins and his associates have utilized this method to compare the carcinogenic potency of a number of polycyclic hydrocarbons (16). Their conclusions have been generally similar to those obtained by others using skin-painting techniques. Nevertheless, penetration of skin by hydrocarbons depends upon molecular structure (3), a property which makes interpretation of early experiments very difficult. The method first used by the Huggins group, and since then by others, does not suffer as greatly from this limitation.

CONCLUSIONS

On the basis of the present experiments it appears that the most efficient method to treat the mammary glands with polycyclic carcinogens is to use either a natural oil or Tween 60 as a solvent. The concentration of carcinogen in the test meal should be as high as possible. Under these conditions deposition of carcinogen in the mammary fat pad is optimal. In some mouse strains the concentration of carcinogen in the mammary tissue after remote introduction into the stomach is as high as that achieved in the skin after direct application (3). In Sprague-Dawley rats uptake of carcinogen by the mammary glands after feeding is much more efficient than uptake by the skin after direct application (3, 7).

The early work with mice, conducted with strains A, C3H, and C57/St, indicated that deposition of BP was much more efficient in rat fat than in mouse fat. It was reasonable, therefore, to assume that the difference in mammary carcinogenesis between these species was due in large part to differential deposition of carcinogen in the mammary fat pad. Subsequent work, however, has demonstrated that many strains of mice are characterized by efficient transport of orally administered BP to the fat. In these cases, resistance to hydrocarbon-induced mammary carcinogenesis is apparently due to metabolic factors or else to poor movement of hydrocarbon from the fat pad to the epithelial cells. An analysis of milk from lactating females may further elucidate this problem.

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9. DAO, T. L. The Role of Ovarian Hormones in Initiating the


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