The Influence of Solvents upon the Effectiveness of Carcinogenic Agents*

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In considering the explanation of the recognized influence of the solvent upon the carcinogenic effectiveness of a dissolved carcinogen we have focussed our attention upon the physical factors which affect the availability of the carcinogen to the tissues in situ. An attempted formulation (see Discussion) of a possible mechanism of transfer from the solvent to the nearby affected tissue suggested that the relative solubility of a carcinogen in solvent and in serum might be of importance. Therefore, we have investigated the relative solubility distribution of benzpyrene in serum and in a number of lipoid solvents for which reliable animal data (8) on tumor incidence have been obtained.

EXPERIMENTAL PROCEDURE

Two-tenths per cent solutions of benzpyrene were prepared by dissolving 5 mgm. of commercial benzpyrene (S.A.F. Hoffman-LaRoche and Co.) in 2.5 ml. of each of the following solvents: cetane, cetane plus cholesterol (1.9 per cent), hydrous lanolin, lard residue, anhydrous lanolin, lard filtrate, sesame oil, olive oil, tricaprylin, and tricaprylin plus 3 per cent cholesterol. Lard filtrate, lard residue, and lanolin, which are not liquid at room temperatures, were warmed in hot water until they liquefied in making up the benzpyrene solutions. The lard filtrate, lard residue, and lanolin, which are not liquid at room temperatures, were warmed in hot water until they liquefied in making up the benzpyrene solutions. The lard filtrate and lard residue were obtained from a sample of commercial lard ("Laurel-Leaf" brand) by filtering through a coarse filter paper at 38° C., following the same procedure as was used by Leiter and Shear (8). The other solvents used were of commercial grade.

The sera used were pooled samples obtained from the Central Laboratories of the University of California Hospital. Ten samples of sera were obtained at different times and were non-uniform; that is, each "normal" sample consisted of sera from "fasting patients," from non-fasting patients, and from jaundiced patients. One sample was pooled sera from jaundiced patients only and another was from non-fasting patients.

Three-tenths milliliters of the 0.2 per cent solution of benzpyrene in any of the solvents mentioned above (the concentration-volume relationship used by Leiter and Shear in their animal experiments) were added to 3 ml. of serum in a 15 ml. centrifuge tube. Here again the solutions in lard filtrate, lard residue, and lanolin had to be warmed in a water bath until liquefied in order to measure the required volume. The lipid layer containing benzpyrene was dispersed into the serum mechanically with a glass plunger at frequent intervals from 24 to 48 hours to insure equilibrium distribution of benzpyrene between the two phases.* The tube was then centrifuged until the two layers separated completely. To extract the benzpyrene the serum was passed through a fine sintered-glass filter and then 1 ml. was transferred to a glass-stoppered 10 ml. volumetric flask to which was added 5 ml. of octane. The flask was then mounted in a mechanical shaker and the contents were intermixed (avoiding emulsification) for 5 hours or more until equilibrium distribution of the

*We wish to thank Mrs. Tillie Leake and Miss June Willard of the University of California Central Laboratories for collecting the serum samples for us.

†We wish to express our indebtedness to Dr. J. L. Hartwell and Dr. M. J. Shear of the National Cancer Institute, and to E. I. du Pont de Nemours and Co. for supplying us with samples of cetane.
benzpyrene was assured. The distribution coefficient favored the octane so markedly that only one such extraction was usually necessary.

**Spectrophotometric Measurement of Benzpyrene in Octane**

The absorption spectrum of the octane extract of the serum containing the benzpyrene was measured in the ultraviolet in cells 1 cm. in size with a Beckman spectrophotometer. In all cases, with the exception of sesame oil, the spectra were identical with that of benzpyrene over the entire range of spectrum from 390 m\(\mu\) to 230 m\(\mu\). The prominent absorption bands at 296 m\(\mu\) and 383 m\(\mu\) were used for calculations of concentrations, using the measured extinction coefficients of the sample of benzpyrene employed for these experiments.

Fig. 1 shows typical absorption spectra of the octane extracts for typical experiments with cetane, sesame oil, lard residue, and olive oil. It may be observed in Fig. 1 that only in the case of sesame oil is the spectrum of the benzpyrene distorted by "background" absorption. This variable background absorption introduces an error in the measurement of the concentration of benzpyrene transferred to the serum obviously such as to make the recorded values err toward high values. This "background" absorption has been identified as sesamin. Because of the demonstrated synergistic action of sesamin (6) combined with its interesting solubility properties, **in vivo** experiments have been initiated to investigate the possibility that sesamin may influence the effectiveness of sesame oil as a vehicle for carcinogenic hydrocarbons.

**RESULTS**

The third column in Table I gives the equilibrium concentrations of benzpyrene in serum. The corresponding distribution coefficients of benzpyrene between the solvents and the serum are presented in the next column. By distribution coefficient is meant the equilibrium ratio, \(C_{\text{solv.}}/C_{\text{serum}}\), where \(C_{\text{solv.}}\) is the concentration of benzpyrene in the solvent and \(C_{\text{serum}}\) is the concentration of benzpyrene in serum.

In Table I it may be seen that for each sample of serum there is a consistent sequence of the distribution coefficients for the different solvents: e.g., cetane < lard residue < lard filtrate < tricaprylin < olive oil. Because the absolute values of the distribution coefficients for a given solvent vary with the sample of serum employed, it is necessary to reduce the values to a standard level for each serum in order to make a quantitative comparison of the relative order of the sequence, independent of the serum sample. This has been done by selecting as a standard, the distribution coefficient for a solvent common to the...
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<table>
<thead>
<tr>
<th>Table I: Equilibrium Concentrations of Benzpyrene between Selected Lipoid Solvents and Samples of Sera in vitro and Distribution Coefficients of Benzpyrene between These Solvents and Sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum sample</td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td>#1. Normal*</td>
</tr>
<tr>
<td>#2. Jaundice</td>
</tr>
<tr>
<td>#3. Non-fasting</td>
</tr>
<tr>
<td>#4. Normal</td>
</tr>
<tr>
<td>#5. Normal</td>
</tr>
<tr>
<td>#6. Normal</td>
</tr>
<tr>
<td>#7. Normal</td>
</tr>
<tr>
<td>#8. Normal</td>
</tr>
<tr>
<td>#9. Normal</td>
</tr>
<tr>
<td>#10. Normal</td>
</tr>
</tbody>
</table>

*See text: Experimental procedure, 2nd paragraph.

**Note:** The concentration in the solvent, Csolvent, is calculated from the difference of the original amount of benzpyrene in the solvent and that transferred to the serum.

different serum samples. Since lard residue is common to more experiments than any other solvent, it is taken as the best choice for a standard of reference of unit distribution coefficient. The distribution coefficients of the other solvents relative to the standard are presented in column 4 of Table II. Corresponding data are presented in column 5 using tricaprylin as the standard. In column 6 olive oil served as the standard. These data are presented graphically in Fig. 2. The reduced data are seen to appear in the same sequence independent of the serum samples.

**DISCUSSION OF RESULTS**

Table III gives the relative order of carcinogenic effectiveness of benzpyrene in lipoid solvents compiled from the literature. Because the results tabulated in Table III and in Figs. 3a and 3b are taken from experiments done elsewhere with solvents from different sources and with different strains of animals of different susceptibilities, it would be unjustifiable to attempt to derive a quantitative correlation between the coefficients of distribution in Table II and the tumor incidences (from Table III) associated with all of the solvents. However, the results with the synthetic solvents, cetane and tricaprylin, may be compared directly with reasonable accuracy. Lard residue and lard filtrate may be compared relative to each other. Although there may be considerable variation of the carcinogenic effectiveness of the benzpyrene in lards from different sources, the lard filtrate has been found in each case to be more effective than the lard residue (8). The relative carcinogenic effectiveness of
### Table II: Relative Distribution Coefficients Independent of Serum

<table>
<thead>
<tr>
<th>Serum sample</th>
<th>Solvent containing benzpyrene (mgm./% benzpyrene)</th>
<th>Distribution coeff. (K)</th>
<th>Lard residue</th>
<th>Tricaprylin</th>
<th>Olive oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1.</td>
<td>Lard filtrate 186</td>
<td>1.24</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Lard residue 150</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#2.</td>
<td>Lard filtrate 150</td>
<td>1.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lard filtrate 150</td>
<td>1.25</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lard residue 120</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#3.</td>
<td>Lard residue 135</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lard residue 135</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anhydrous lanolin 157</td>
<td>1.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hydrous lanolin 100</td>
<td>0.74</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>#4.</td>
<td>Lard residue 151</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sesame oil 390</td>
<td>2.58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#5.</td>
<td>Cetane 56</td>
<td>0.43</td>
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<tr>
<td></td>
<td>Cetane 70</td>
<td>0.54</td>
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<tr>
<td></td>
<td>Lard residue 137</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lard residue 127</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#6.</td>
<td>Olive oil 341</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Olive oil 333</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sesame oil 250</td>
<td>0.74</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Sesame oil 347</td>
<td>1.02</td>
<td></td>
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<tr>
<td>#7.</td>
<td>Olive oil 354</td>
<td>1.82</td>
<td>1.00</td>
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<tr>
<td></td>
<td>Sesame oil 265</td>
<td>1.36</td>
<td>0.75</td>
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<tr>
<td></td>
<td>Cetane 90</td>
<td>0.46</td>
<td>0.25</td>
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<tr>
<td></td>
<td>Lard residue 194</td>
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<tr>
<td>#8.</td>
<td>Cetane 50</td>
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</tr>
<tr>
<td></td>
<td>Cetane 47</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cetane+cholesterol 57</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cetane+cholesterol 57</td>
<td>1.00</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>#9.</td>
<td>Cetane 34</td>
<td>0.24</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tricaprylin 138</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tricaprylin 144</td>
<td>1.00</td>
<td></td>
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<tr>
<td></td>
<td>Anhydrous lanolin 123</td>
<td>0.87</td>
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<tr>
<td></td>
<td>Hydrous lanolin 95</td>
<td>0.67</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>#10.</td>
<td>Cetane 39</td>
<td>0.35</td>
<td>0.24</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tricaprylin 160</td>
<td>1.45</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tricaprylin+choles. 170</td>
<td>1.55</td>
<td>1.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lard residue 110</td>
<td>1.00</td>
<td>0.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lard filtrate 126</td>
<td>1.15</td>
<td>0.79</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Benzopyrene in tricaprylin and lard filtrate cannot be compared because the tumor incidence overlaps (see Table III) in comparable experiments with different samples. Sesame oil is so markedly effective a vehicle (8, 18) (see Figs. 3a and 3b) that it may be taken as relatively higher than the others in the carcinogenic series of solvents regardless of its source. The values of the relative distribution coefficient of sesame oil have been semi-quantitatively corrected for the effect of the unique "background" absorption on the measured values. The data for relative effectiveness (Table III and Fig. 3a) of benzopyrene in the solvents cetane, lard residue, lard filtrate, tricaprylin (specimens A, B, C, and D), and sesame oil (A) as vehicles, are particularly reliable because they have been determined in the same laboratory (8) with unusually rigorous experimental techniques.

Thus, all but olive oil and lanolin have been tested under relatively comparable conditions. Olive oil is well known to be an effective solvent (11, 12) and anhydrous lanolin has been reported as an ineffective solvent (13).

Comparison of Table II and Fig. 2 with Table III shows a correlation between the solvent-serum transfer of benzpyrene and the relative carcinogenic effectiveness of the benzpyrene in the following solvent vehicles which have been adequately tested in animals under comparable conditions: cetane, lard residue, lard filtrate, tricaprylin, and sesame oil (8). The correlation of data derived from solvent-serum distribution of benzpyrene with tumor incidence in animal experiments indicates that this simple physical property may be of major importance in explaining the effects of a solvent upon the carcinogenic efficiency of a dissolved carcinogen.

That a relationship, although complex and difficult to evaluate, should exist between solvent-serum distribution and solvent effectiveness is compatible
with considerations of the physical availability of the carcinogen to the affected tissue. We have attempted to formulate the action of the carcinogen, benzpyrene, in terms of the factors affecting the concentration-time relationship of the benzpyrene on the cells of the tissues affected. The analysis is based upon modifications of the treatment as given

The $C$, $T$ functions that have been found to fit experimental data do not necessarily describe a mechanism, but be that as it may, the results always can be expressed in accord with a $C$, $T$ function in which $C$ and $T$ produce mathematically inverse effects.

Thus, if a cell is exposed for a total time $T$ to

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Relative carcinogenic effectiveness</th>
<th>Benzpyrene dose</th>
<th>subeutaneous, mg/m</th>
<th>Tumor incidence</th>
<th>Reference</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetane C$<em>{16}$H$</em>{34}$ M.P. 16 C.</td>
<td>Very low</td>
<td>0.05-0.1</td>
<td>0-4</td>
<td>(8)</td>
<td></td>
<td>Two experiments, 50 mice each, “A” strain.</td>
</tr>
<tr>
<td>Lard residue specimen D</td>
<td>Low</td>
<td>0.065-0.1</td>
<td>4-14</td>
<td>(8)</td>
<td></td>
<td>Four experiments, 50 mice each, “A” strain.</td>
</tr>
<tr>
<td>Anhydrous lanolin</td>
<td>(Methylcholanthrene)</td>
<td>0 ?</td>
<td></td>
<td>(13)</td>
<td></td>
<td>Methylcholanthrene painted on the surface; probably less effective than subeutaneous injection. Swiss strain.</td>
</tr>
<tr>
<td>Tricaprylin C$<em>{27}$H$</em>{50}$O$_{4}$ M.P. 6-9 C.</td>
<td>Intermediate</td>
<td>0.3</td>
<td>38-46</td>
<td>(17)</td>
<td></td>
<td>30 mice of Glaxo FF strain.</td>
</tr>
<tr>
<td>Specimen A</td>
<td>0.3</td>
<td>84-94</td>
<td>30 mice of Glaxo FF strain.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specimen B</td>
<td>0.3</td>
<td>84-94</td>
<td>30 mice of Glaxo FF strain.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specimen C</td>
<td>0.3</td>
<td>84-94</td>
<td>30 mice of Glaxo FF strain.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specimen D</td>
<td>0.3</td>
<td>84-94</td>
<td>30 mice of Glaxo FF strain.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tricaprylin</td>
<td>0.3</td>
<td>84-94</td>
<td>30 mice of Glaxo FF strain.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tricaprylin with 3%</td>
<td>0.3</td>
<td>84-94</td>
<td>30 mice of Glaxo FF strain.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cholesterol</td>
<td>0.3</td>
<td>84-94</td>
<td>30 mice of Glaxo FF strain.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lard filtrate from:</td>
<td></td>
<td></td>
<td></td>
<td>(17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lard specimen A</td>
<td>0.1</td>
<td>30</td>
<td>20 mice</td>
<td>(8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lard specimen B</td>
<td>0.1</td>
<td>80</td>
<td>20 mice</td>
<td>(8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lard specimen C</td>
<td>0.1</td>
<td>80</td>
<td>20 mice</td>
<td>(8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lard specimen D</td>
<td>High</td>
<td>0.65</td>
<td>84</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lard specimen D</td>
<td>High</td>
<td>0.65</td>
<td>84</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sesame oil (A)</td>
<td>Very high</td>
<td>0.05</td>
<td>68</td>
<td>(8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sesame oil (B)</td>
<td>Very high</td>
<td>0.05</td>
<td>68</td>
<td>(8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olive oil (A)</td>
<td>Very high</td>
<td>0.05</td>
<td>68</td>
<td>(8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olive oil (B)</td>
<td>Very high</td>
<td>0.05</td>
<td>68</td>
<td>(8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The relatively higher dosages used by Morton and Mider (9) to produce the same effectiveness obtained by Leiter and Shear (8) may be attributed to the different strains of mice used. The former used a resistant strain, the latter a susceptible strain.

† Data indicate high effectiveness, but no data is available for relative quantitative comparison.

by A. J. Clark (2) of the action of drugs and other agents in producing biological effects.

If a concentration $C$ of an agent acts for a time $T$ and an effect $E$ is produced there is a relationship between $C$ and $T$ which, in simple cases, is approximated by $C \times T = \text{constant}$. In all cases reported (2) the $C$, $T$ function has been adequately expressed in the form: $(C - C_m)^n(T - T_m) = \text{constant}$, where:

- $C_m$ is the minimum or threshold concentration below which, even acting for an infinite time, $C_m$ will not produce the effect;
- $T_m$ is the minimal time below which even the uppermost concentration attainable cannot produce the effect;
- $n$ is an arbitrary constant which has been found to vary from 0.3 to 8.0 for different agents and effects.

\[ \int_0^T C dt < P_m \] (where $P_m$ is the minimal value of the $C$, $T$ function which will produce the effect) then the effect will not occur.

If the $\int_0^T C dt = P_m$ the effect will occur.

\[ \int_0^T C dt > P_m \] the effect will occur and the quantity of the effect $E$ will be a function (probably not linear) of the magnitude of $\int_0^T C dt$ i.e., $E = f(P)$.

In attempting to determine whether "physical factors" may be important in determining the ef-
fectiveness of different lipoid solvents upon the incidence of tumors induced by a single subcutaneous injection of benzpyrene in these solvents, we can examine the mechanism of the action of benzpyrene in the light of these concepts. The problem requires that we try to evaluate directly or indirectly the concentration of benzpyrene in the affected tissue at all times during the arbitrary time interval chosen to test appearance of tumors. In practice the quantity of the effect

optical density of a photographic plate is a measurable effect $E$ resulting from incident light energy of intensity $I$, twice the incident energy does not produce twice the optical density, which varies rather with the logarithm of the energy according to the well known H-D relationship.

To visualize the meaning of the $C, T$ function in the cells subjected to the tumor-producing carcinogen, consider Fig. 4, which represents schematically (not to scale) an idealized physical description of the problem. It is assumed:

1. The lipoid solvent containing benzpyrene is immersed in the interstitial fluid at the site of injection and stays there in significant part throughout the time $T$.

Although the lipoid solvents may stay at or near the site of injection for several months (5, 12) their area/volume ratio may alter. The area/volume variable, neglected here, could be a major influence in solvent effectiveness. We have observed, using ultraviolet fluorescence, that a lipoid solvent and its content (fluoranthene) injected into mice was present in the neighboring subcutaneous fat and in other fat depots in finely dispersed microscopic droplets after many weeks (unpublished data).

![Distribution coefficients of various solvents relative to lard residue, tricaprylin, and olive oil.](image-url)
2. The cells of the adjacent tissue are exposed to a variable concentration of benzpyrene during the time of its distribution and excretion, dependent upon various factors. One of the factors affecting the value of the effective product of the concentration and the time it acts, i.e. \( \int Cdt \), will be the relative rate of transfer of the benzpyrene between the solvent and the interstitial fluid, between the interstitial fluid and the cells of the adjacent tissue, and between the interstitial fluid and the serum (see Fig. 4). It may be assumed that equilibrium between solvent and interstitial fluid takes place relatively rapidly across the liquid-liquid boundary.

3. The concentration gradient and the rate of transfer of benzpyrene across the endothelial barrier between the interstitial fluid and the serum is a determining factor of the \( \int Cdt \) in the adjacent tissue. Because the carcinogen is removed rapidly from serum there will be expected a larger gradient and rate of flow of the carcinogen to the serum from the interstitial fluid than to the adjoining tissue cells. The greater the transfer of the carcinogen from the solvent to the extracellular fluid the more pronounced the effect the differential rate would accordingly have on the value of \( \int Cdt \) in the tissue. In addition, the cell barrier, in the transfer from interstitial fluid to the adjacent tissue cells, is probably more difficult to penetrate than the endothelial barrier. It follows that a greater rate of transfer from solvent to serum may result in a lesser net effect on the tissue.

4. The rate of transfer from the solvent to the interstitial fluid is a direct function of the distribution coefficient of the benzpyrene for these two solvents, and the distribution coefficient for the solvent and serum will approximate that for solvent and interstitial fluid.

Accepting the mechanism represented in Fig. 4 and the assumptions stated above, the rate of transfer of benzpyrene from solvent to interstitial fluid and serum will approximate that for solvent and interstitial fluid.

\[ A \text{ state of equilibrium as represented by the distribution coefficient for two solvents does not necessarily correlate with the relative rates of transfer. If it is assumed that there are only quantitative not qualitative changes in the nature of the serum-solvent boundary in going from one lipoid solvent to another (such as tricaprylin to cetane) the assumption of correlation of rate and equilibrium data is of the order of validity of correlation of rate of solution with solubility.} \]
fer from solvent to interstitial fluid may be functionally measured by evaluating the coefficient of distribution of benzpyrene between the solvent and serum. If the coefficient of distribution varies significantly with the solvent, then the \( \int_0^t C dt \) in the cells of the adjacent tissue will be affected and thus the solvent could influence the effectiveness of the carcinogen. That the solvent would have an influence, is obvious from hypothetical extreme cases: (a) the solvent holds the benzpyrene so effectively that practically none escapes, then the \( \int_0^t C dt \) \( = 0 \), and obviously no effect can occur; (b) again, if the benzpyrene escapes from the solvent very slowly and is carried away by the serum rapidly the concentration at the adjacent tissue may be always less than the threshold concentration, then \( \int_0^t C dt < P_m \); (c) likewise on the other extreme, if the escape and carry-off is so rapid that the high concentration is not present for the threshold time value, then \( \int_0^t C dt < P_m \). This case is exemplified by using serum or an effective water-like solvent or by using a solubilizing agent for the carcinogen (3). It follows that there is an intermediate rate of escape which will lead to an optimal \( C, T \) function, i.e. \( \int_0^t C dt = P_{optimal} \).

The question has been raised by Dickens (3), Dickens and Weil-Malherbe (4), and Leiter and Shear (8) as to whether the anti- or pro-carcino-

![Diagram](https://cancerres.aacrjournals.org/)

**Fig. 4.**—Schematic representation of dynamic mechanism determining effective concentration-time relationship of injected carcinogen in adjacent tissue upon which it acts.

...
The "sensitization of skin by carcinogenically in-
active methycholanthrene to subsequent carcino-
genesis" reported by the same authors (15), may
logically follow then as a result of the relatively
minor action of a relatively high concentration act-
ing for a short time. Evidence of such threshold
biological actions after treatment with benzpyrene
in lanolin, has been described in their report; the
observed reduction in the size and number of the
sebaceous glands, the increase in keratin, and the
appearance of precancerous papillomas after the
23rd week of treatment (7, 13). It seems doubtful
to us that this experimental evidence may be taken
as proof of the existence of otherwise unknown
agents which have biochemical significance in the
mechanism of carcinogenesis by the carcinogenic
hydrocarbons.

Other experimental evidence has been presented
that the variation in the incidence of tumors due to
the influence of a solvent resides in the effective-
ness of the carcinogen in reaching the direct site
of the tumor and staying there long enough at an
adequate or at a most effective concentration. Be-
sides the work of Peacock and Beck (12), Cheva-
litier, Denoix, and Maurel (1) have observed that
the intramuscular injection of benzpyrene (0.5
mgm. in 0.5 ml. of olive oil), which induced a
high incidence of tumors in white rats, remained
localized around the site of injection. Conversely,
in guinea-pigs the benzpyrene diffuses away in 4
days and no tumors ensue.

The theory advanced here would predict that,
other factors, e.g. solvent absorption and detoxifi-
cation, local tissue reaction, and redistribution being
equal, a low incidence of tumors locally is more
likely to be accompanied by an increase of tumors
elsewhere in the body than vice versa because a
rapid absorption from the site favors the probability
of a high concentration-time product elsewhere
—either in the circulation or in distant organs.
Leukemias and liver tumors have been reported under
these circumstances (10).

Although the physical factor of solubility ap-
ppears to have a significant influence on the carci-
gen-vehicle effectiveness, one cannot dismiss the
possibility that chemical factors may be associated
with the relative effectiveness of lipid solvents as
vehicles.

SUMMARY

The coefficients of distribution of benzpyrene
between 8 lipid solvents, (which have been used
experimentally in animals as vehicles for this car-
cinogen), and serum have been measured. The
relative values of the coefficients of the lipid sol-
vents appear as a spectrum in a graded series from
0.2 to 2.0 which correlates closely with the recorded
variation in carcinogenic effectiveness of benzpyrene
when injected subcutaneously in these solvents as
vehicles.

The close correlation of the solvent-serum dis-
tribution with tumor incidence indicates that this
physical property is important in explaining the
observed influence of solvents upon carcinogenic
response and must be taken into account as a vari-
able before attributing the observation to the
chemical properties of the solvents or of assumed
"co-carcinogenic" constituents.

A mechanism has been formulated to describe
the influence of the solvent as a vehicle affecting
the availability of the carcinogen as an agent which
acts upon the adjacent affected tissue cells. The
effectiveness is then dependent upon an integrated
concentration-time function of the carcinogen at
the site of its action. In terms of this mechanism,
solvent-serum solubility distribution should be an
important factor in determining the availability
function.

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