A hypothesis formulated by Otto Schmidt (21) to account for the carcinogenic activity of chemical substances has been widely exploited during the last 15 years (for review see [19]).

The theory is based on the assumption that a very important step in the production of a cancer is an interaction between cellular constituents and a zone of the hydrocarbon which is particularly rich in \( \pi \) electrons.

The proposal of Schmidt has received confirmation from two kinds of investigations. On the one hand, the complex between carcinogenic substance and proteins has been demonstrated experimentally (1, 10, 11, 13, 14, 23). On the other hand, theoretical investigations have shown, for example, that there is a parallel between the electronic charge of a certain region of angular benzantracene derivatives and the carcinogenic activity of these molecules (4, 19).

Since carcinogenic molecules in general have a bond with a high bond order (5, 19) (which is often called the K region) (19), it has seemed reasonable to suppose that a necessary condition for an aromatic derivative to be carcinogenic is that it have a strong tendency to add to cellular proteins by one of its bonds. However, Woodhouse (24) has shown that certain noncarcinogenic hydrocarbons are also fixed on tissue. Thus, perhaps the formation of the complex is not as important as the kind of complex which is formed (15).

As the nature of the complex is a problem which is very difficult to investigate experimentally, we propose in this paper to suggest from theoretical considerations different possibilities which may be then correlated with biological activity.

Theoretical Study of the Kinetics of the Fixation of Conjugated Hydrocarbons

Bhargava and Heidelberger (1) have observed that 25 per cent of the carcinogenic hydrocarbon 1,2,5,6-dibenzanthracene which is bound to proteins is transformed into 2-phenylphenanthrene-3,2'-dicarboxylic acid.

Thus, it seems reasonable to believe that at least 25 per cent of the 1,2,5,6-dibenzanthracene has reacted with the proteins by its mesophenanthrenic bond (K region) (19). This is natural from the quantum chemical point of view, since the mesophenanthrenic bonds have the highest bond order (Chart 1) and thus are very active in addition reactions.

Heidelberger and Moldenhauer (10) have shown that 1,2,3,4-dibenzanthracene, which is noncarcinogenic, is also fixed to cellular proteins and quantitatively even more than 1,2,5,6-dibenzanthracene. How 1,2,3,4-dibenzanthracene is bound is not known. The bond orders are all considerably less than 0.778. However, there are a pair of para (mesoanthracenic) positions with large free valence (Chart 2) (one such pair has been called the L region [18]). It is well known that such a region is very reactive toward addition reactions. The diene synthesis, or Diels-Alder reaction, is a well known example of such an addition. It
is thus very tempting to assume that addition to the protein takes place at this pair of positions.

In this paper we shall make this hypothesis.

Thus, one can imagine that an aromatic hydrocarbon which is in contact with a protein in vivo can be bound by addition either to one of the mesophenanthrenic bonds or to a pair of mesoanthracenic positions. Comparable reactions are known in chemistry. For example, bromine adds to butadiene both 1-2 and 1-4, thus:

\[
\begin{align*}
\text{CH}_2 = \text{CH} - \text{CH} = \text{CH} + \text{Br}_2 & \rightarrow \text{Br} - \text{CH}_2 - \text{CHBr} - \text{CH} = \text{CH}_2 \\
& \rightarrow \text{Br} - \text{CH}_2 - \text{CH} = \text{CH} - \text{CH}_2\text{Br}
\end{align*}
\]

It may be thought that this hypothesis is not in agreement with the concept of the L region (18). The presence in a molecule of a pair of para-positions which can react by addition has been observed to be unfavorable for the carcinogenic property of the molecule. It is perhaps tempting to explain this result by suggesting that the addition has occurred with substances other than cellular proteins, and thus the necessary first step of the rate constant \( k \) associated with each of the bonds of the hydrocarbon:

\[
k = \sum_i k_i.
\]

The potential barrier \( U_i \) corresponding to \( k_i \) then can be written as:

\[
k = \sum_i A_i e^{-U_i/RT}.
\]

The multiplicators \( A_i \) include the partition functions. These terms are unfortunately not known for the reaction which interests us here. There is a linear relation between the log of the rate constant and the free valence of the localization energy for several reactions of these hydrocarbons—chloromethylation (19), methylation (6), nitration (7), exchange reactions (8)—which seems to indicate that the term \( A_i \) is generally constant for a given reaction (6). We will assume that is so here. Then the total rate constant can take the various forms:

\[
k = a \sum_i e^{-U_i/RT} = \frac{b}{c} \sum_i e^{-U_i/RT} = c \sum e^{-\frac{m_i \beta}{RT}}.
\]

Here \( U_i \) is the energy of ortholocalization and \( m_i \beta \) is the important variable of this quantity. According to Wheland (22), the only varying part of the potential barrier \( U_i \) is the contribution \( U_{\pi} \) due to the \( \pi \) electrons. This is called the localization energy and can be readily expressed in terms of the resonance integral \( \beta \), which is a parameter in the molecular orbital method.

In a similar way the rate constant of the reaction which proceeds by addition across a pair of para-positions can be given the form:

\[
k' = c' \sum e^{-m' \beta/RT}.
\]

The values of \( m \) and \( m' \) can be calculated very conveniently by the molecular orbital method.

There exist at least two different ways of calculating these quantities according to a procedure either by Wheland (22) or by Muller, Pickett, and Mulliken (16). Nagata, Fukui, Yonezawa, and Tagashira (17) have stated that the order of the reactivities of several hydrocarbons depends

\[\text{on April 14, 2017. © 1958 American Association for Cancer Research. cancerres.aacrjournals.org Downloaded from cancerres.aacrjournals.org on April 14, 2017. © 1958 American Association for Cancer Research.}\]
upon which of these two methods is used to calculate the localization energy. However, two of us have shown that the result of the Japanese workers is due to the introduction of supplementary approximations in the calculations. The exact calculation carried out by both methods leads to the same result (3). More recently, the problem has been reconsidered by the Japanese in more general terms, and our result has been confirmed (9). We will thus only use the simpler Wheland method to calculate the localization energy. The values for $m_\alpha$ and $m^\prime_\alpha$ for a number of molecules already exist in the literature.

We cannot, of course, theoretically treat the kinetics of the fixation of the hydrocarbons by the proteins after the substances have been introduced in a living organism. All we can do is to treat the following greatly simplified model which may, we hope, throw some light on the metabolism of the hydrocarbons.

We imagine that a certain quantity of hydrocarbon $A$ is introduced into the animal, and we assume that (a) the substance can be fixed by addition to a cellular protein present in considerable excess either across a bond or across a pair of para-positions; (b) the substance does not react with other kinds of molecules; (c) the complexes which are formed are afterward destroyed by a mechanism which is similar to the one which the organism uses to eliminate exogenous material.

Schematically, this could be represented as:

\[
\begin{align*}
\text{A} & \xrightarrow{k} \text{C} & \xrightarrow{k_\alpha} \text{M} \\
\text{A} & \xrightarrow{k^\prime} \text{C}' & \xrightarrow{k_\alpha} \text{M}'
\end{align*}
\]

where $k$ is the total rate constant for the production of the complexes $C$ that result from fixation by the bonds, $k^\prime$ the constant corresponding to the formation of complexes $C'$ that result from addition by the vertices, $k_\alpha$ the rate of decomposition of complex $C$ and of complex $C'$.

The quantities of the complexes $C$ and $C'$ at the beginning of the reaction are zero and are also zero at infinite reaction time. These quantities pass through maxima which can be readily shown to be respectively proportional to:

\[
B = \frac{k}{k_\alpha - (k + k')} \times \left[ \left( \frac{k + k'}{k_\alpha} \right)^{-k/k_\alpha - k'} - \left( \frac{k + k'}{k_\alpha} \right)^{-k/k_\alpha - k'} \right]
\]

and:

\[
S = \frac{k'}{k_\alpha - (k + k')} \times \left[ \left( \frac{k + k'}{k_\alpha} \right)^{-k/k_\alpha - k'} - \left( \frac{k + k'}{k_\alpha} \right)^{-k/k_\alpha - k'} \right]
\]

The total amount of bound hydrocarbon is evidently proportional to:

\[
T = B + S
\]

Table 1 assembles for a number of hydrocarbons the values of $B$, $S$, and $T$, some indications about the carcinogenic activity, and the maximum quantity $Q$ fixed by soluble proteins (in mg/mg hydrocarbon/protein) obtained by Heidelberger and Moldenhauer (10). The notation of increasing carcinogenic activity ($\pm, +, ++, +++$, $++++$) is not very precise, since this activity can, of course, vary a great deal depending on the line of the animal, the concentration of hydrocarbon, the method of application, etc. Likewise, the values of $B$, $S$, and $T$ are only reasonable figures, since in the calculation of $k$ and $k'$ there is an arbitrary constant which remains undetermined.

To obtain plausible values we have taken empirically:

\[
k_i = k^\prime_i = 1 \quad \text{for} \quad m_i = 1.15 \quad \text{and} \quad m^\prime_i = 3.65,
\]

and we have taken several reasonable values for $k_\alpha$.

The table gives the values of $B$ and $S$ for $k_\alpha = 300$ (first line) and $k_\alpha = 3000$ (second line). The qualitative conclusions do not differ greatly for this rather considerable variation of $k_\alpha$.

**DISCUSSION**

From a careful examination of Table 1 it will be seen that the hydrocarbons naturally fall into several different groups.

The first group includes the molecules for which $B$ (and consequently $T$) is rather large ($>0.1$ for $k_\alpha = 300$), while $S$ is nearly zero. This group includes the substances which should readily be fixed to proteins by one of its bonds. It is in this group that the carcinogenically very active compounds are found.

The second group is made up of hydrocarbons for which both $B$ and $S$ (and thus $T$) are rather large ($>0.1$). These molecules should theoretically add to a protein either by addition to a bond

These values have been assembled in the *Dictionary of Theoretical Properties Descriptive of Molecules*, edited by the Mathematical Institute (Oxford) and the Centre de Mécanique Ondulatoire Appliquée (Paris).
or to a pair of para-positions. This group contains the substances which are rather active carcinogens.

The third group is made up of noncarcinogenic hydrocarbons for which $B$ is small and $S$ (thus $T$) is large. These hydrocarbons should add to proteins, but this addition should only take place by a pair of para-positions.

The fourth class includes those molecules for which both $B$ and $S$ (and consequently $T$) are quite small. These molecules are noncarcinogenic and should not fix to proteins if our theoretical predictions are correct.

If our hypotheses are valid, then $Q$, which represents the proportion of hydrocarbon which is experimentally found to be fixed to protein, should vary like the theoretical quantity $T$ and not like $B$.

Unfortunately, there are not enough experimental results available to establish whether or

### TABLE 1

<table>
<thead>
<tr>
<th>MOLECULES</th>
<th>CARCINOGENIC POWER</th>
<th>B</th>
<th>S</th>
<th>T</th>
<th>Q†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dibenzo[a,j]pyrene</td>
<td>++</td>
<td>0.94</td>
<td>~0</td>
<td>0.94</td>
<td>0.94</td>
</tr>
<tr>
<td>Dibenzo[ad,je]pyrene</td>
<td>−</td>
<td>0.87</td>
<td>0</td>
<td>0.87</td>
<td>0.87</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>++</td>
<td>0.80</td>
<td>~0</td>
<td>0.80</td>
<td>30</td>
</tr>
<tr>
<td>Dibenzo[a,e]pyrene</td>
<td>++ (?</td>
<td>0.80</td>
<td>~0</td>
<td>0.80</td>
<td>0.80</td>
</tr>
<tr>
<td>Pyrene</td>
<td>−</td>
<td>0.60</td>
<td>0</td>
<td>0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>−</td>
<td>0.42</td>
<td>~0</td>
<td>0.42</td>
<td>2</td>
</tr>
<tr>
<td>Benzo[e]pyrene</td>
<td>+</td>
<td>0.44</td>
<td>~0</td>
<td>0.44</td>
<td>0.44</td>
</tr>
<tr>
<td>Dibenzo[a,h]pyrene</td>
<td>++</td>
<td>0.25</td>
<td>~0</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Benzo[c]phenanthrene</td>
<td>−</td>
<td>0.14</td>
<td>~0</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>Dibenzo[a]anthracene</td>
<td>+</td>
<td>0.19</td>
<td>0.75</td>
<td>0.94</td>
<td>25</td>
</tr>
<tr>
<td>Dibenzo[a]anthracene</td>
<td>+ (?)</td>
<td>0.19</td>
<td>0.75</td>
<td>0.94</td>
<td>0.94</td>
</tr>
<tr>
<td>Dibenzo[b,h]phenanthrene</td>
<td>−</td>
<td>0.008</td>
<td>~1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>±</td>
<td>0.003</td>
<td>~1</td>
<td>1</td>
<td>0.003</td>
</tr>
<tr>
<td>Naphtho[2,3-a]pyrene</td>
<td>−</td>
<td>~0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dibenzo[a,e]anthracene</td>
<td>−</td>
<td>~0</td>
<td>0.99</td>
<td>0.99</td>
<td>47</td>
</tr>
<tr>
<td>Picene</td>
<td>−</td>
<td>0.07</td>
<td>~0</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Chrysene</td>
<td>−</td>
<td>0.04</td>
<td>~0</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>−</td>
<td>~0</td>
<td>~0</td>
<td>~0</td>
<td>~0</td>
</tr>
</tbody>
</table>


† $Q$ is given in mg of hydrocarbon fixed/mg of protein.
not this result is valid. However, from the data
which are available, the correlation between \( Q \)
and \( T \) is not too bad. \( Q \) varies between 10 and 47
when \( T \) varies between 0.75 and 1 \((k_i = 300)\) and
falls to 2 for \( T = 0.4 \). Thus, one can understand
why the noncarcinogenic 1,2,3,4-dibenzanthracene
\((T = 0.99)\) is fixed to cellular protein even more
than the carcinogenic 1,2,5,6-dibenzanthracene \((T =
0.94)\).

It seems unlikely that there will be a very
exact parallel between \( Q \) and carcinogenic power,
since the third group is made of noncarcinogenic
substances for which \( T \) is large, and consequently
\( Q \) should also be large.

There is the possibility that a better correlation
will exist between carcinogenic activity and the
theoretical quantity \( B \). The correlation cannot
be 100 per cent, since there are some substances
with a high value of \( B \) which are not carcinogenic;
but a study of Table 1 shows that a high value
of \( B \) is a necessary (if not sufficient) condition
that the molecule be an active carcinogenic. The
probability that a conjugated hydrocarbon be
carcinogenic seems to be an increasing function
of \( B \). One of the striking exceptions is the hydro-
carbon anthanthrene. This molecule (cf. Table
1) has a large value of \( B \) and zero value for \( S \),
since it does not seem to have an L region in
the usual way. Perhaps, however, as H. Schmidt
\((20)\) has proposed, addition can take place at
the following positions:

\[
\begin{align*}
\text{Now the values of } B \text{ and } S \text{ are greatly changed and are:} \\
B &= 0.015, \quad \text{and} \quad S = 0.98.
\end{align*}
\]

Thus, anthanthrene now belongs to Group 3,
where the molecules which are noncarcinogenic
are found.

It seems to us that it would be very interesting,
in order to understand the value of the theoretical
discussion of the mechanism of carcinogenesis
which has been presented in this paper, to extend
the elegant work of Heidelberger and his co-
workers. The purpose of these experiments would
be to find out the different ways that an aromatic
hydrocarbon can be bound to protein, as well as
the relative amounts fixed in these different ways.
A part of this program has been undertaken
at the Institut du Radium, and the results will
be reported in due course.

**SUMMARY**

If in giving a particular interpretation to a
“K-L regions” theory it is assumed that aro-
matic hydrocarbons can be fixed to cellular pro-
teins either by a bond or by two nonadjacent
positions, then the experimental observations of
Heidelberger and Moldenhauer can be under-
stood.

The theory which has been proposed in this
paper seems to indicate that it is rather improbable
that there is a clear relation between the total
amount of a substance which is fixed and its
carcinogenic power; however, it remains probable
that a necessary condition for a substance to be
carcinogenic is that a substantial amount is fixed
by one of its bonds to the protein.

Experiments have been suggested which should
make it possible to test the value of the theory.

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Part VI: The Nitration of Aromatic Hydrocarbons; Partial


A Note on the Interaction of Carcinogenic Molecules with Cellular Protein

O. Chalvet, R. Daudel and C. Moser