The Possible Role of Hydrophobic Interactions of Polycyclic Aromatic Hydrocarbons with Protein in Chemical Carcinogenesis

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SUMMARY

Using thermodynamic data for the hydrophobic binding of polycyclic aromatic hydrocarbons to human serum albumin and the $|K|$ values of O. Chalvet ("Mécanique ondulatoire et biologie moléculaire," p. 99, Paris, 1961) as a measure of chemical reactivity, the possible role of hydrophobic interactions of polycyclic hydrocarbons with protein in the process of chemical carcinogenesis has been investigated. It was shown that these interactions are necessary and favorable for the process of chemical carcinogenesis, but that their role is only of secondary importance as compared with the chemical reactivity of the hydrocarbons. The influence of hydrophobic interactions may be due to the induction of a conformational change in a receptor protein. Multiple regression analysis yielded a new complex index which permits the correct grouping of the hydrocarbons considered according to their carcinogenic activity. The concept presented in this paper offers a simple explanation for the drop in carcinogenic activity observed if the hydrocarbons are substituted with polar substituents or bulky alkyl groups, as well as for the interference among hydrocarbons in skin carcinogenesis. The results are in keeping with both the *K-L*-region theory of chemical carcinogenesis and the protein deletion hypothesis.

INTRODUCTION

It is well known that polycyclic aromatic hydrocarbons are solubilized by proteins as a result of rather weak and unspecific (hydrophobic) interactions, and the question has been raised whether such interactions may be of importance for the process of chemical carcinogenesis (1–7; see also ref. 8). Though there are several papers dealing with the solubilization of polycyclic hydrocarbons by proteins (9–26), the interpretation of the results in terms of chemical carcinogenesis presents considerable difficulties. This springs from the fact that such interactions are completely unspecific by nature whereas chemical carcinogenesis seems to be a highly specific process. Thus, physical interactions of hydrocarbons with proteins can only be of secondary importance, and any investigation into the possible role of these interactions must take into account those properties of the hydrocarbons which are more closely related to carcinogenesis. Numerous attempts have been made to find such properties and to correlate them with carcinogenic activity. The most successful approach so far seems to be the *K-L*-region theory of the Pullmans (27–30). According to this theory the crucial phase in chemical carcinogenesis is a chemical reaction of the hydrocarbon with a cellular receptor, most likely a protein,

through the so-called *K*-region, which resembles the 9,10-double bond in the phenanthrene molecule. The hydrocarbons may, however, possess a second active center of the type of the 9,10-*para* positions in the anthracene molecule, which is called the *L*-region.

It is believed that too reactive an *L*-region may draw the hydrocarbon into a different kind of chemical reaction which is incompatible with carcinogenesis. Thus, an active hydrocarbon should have a reactive *K*-region and a rather inactive *L*-region, if such is present. This theory is in excellent agreement with biochemical results on the covalent binding of hydrocarbons with proteins *in vivo* and in full keeping with the protein deletion hypothesis [for a detailed discussion, see, e.g., Pullman (29) and Heidelberger (31, 32)]. For the time being, therefore, it seems justifiable to accept this theory, at least as a working hypothesis, and to use it as a starting point in investigating the possible role of hydrophobic interactions between hydrocarbons and proteins in chemical carcinogenesis. The purpose of the present paper is to carry out such an investigation on the basis of the *K*-*L*-region theory, using thermodynamic data on the binding of polycyclic aromatic hydrocarbons to human serum albumin (23–26). It will be shown that such interactions are indeed involved in the process of chemical carcinogenesis.

STATISTICAL TREATMENT

To solve the problem concerned, it is necessary to investigate the interrelationships between three variables y, x, and z, which characterize respectively the carcinogenic activity, the chemical reactivity, and the hydrophobic interactions with protein of the hydrocarbons in question. In particular, the degree of association between y and z with the effect of x removed and the relationship between y and the combination of x and z have to be estimated. This is accomplished by the usual techniques of partial and multiple correlation and regression analysis (e.g., ref. 33).

All correlation coefficients were transformed to z values according to Fisher before tests of significance were performed (see refs. 34 and 35). Rank correlation coefficients according to Spearman with correction according to Kelly (33) were calculated for all simple correlations in addition to the product-moment correlation coefficients as a control. The agreement between the two sets of coefficients was very good in all cases. This may be considered an indication that the y values are normally distributed. In the following section suitable quantities for the three variables y, x, and z are defined, and the simple correlations between these quantities are established. All data used for the calculations are summarized in Table 1. Included in this table are all hydrocarbons for which values of all three variables were available.

Definition of Three Variables y, x, and z to Characterize Carcinogenic Activity, Chemical Reactivity, and Hydrophobic Binding to Protein of the Hydrocarbons, and Simple Correlations

Carcinogenic activity (y). To arrange the hydrocarbons in order of carcinogenic potency is a very difficult task (for a detailed discussion, see refs. 36–42). Attempts have been made in the past to express carcinogenic activity in terms of certain quantitative indices (43, 44). However, carcinogenic activity cannot be considered a well-defined and characteristic property of chemical compounds. Thus, the use of these indices in comparing the relative potencies of different hydrocarbons is apt to give a greater semblance of accuracy than is justified, owing to the uncertainties inherent in the biological assays. Badger (45) has suggested a much simpler system of grading, according to which the hydrocarbons are divided into five groups of different activity. The potency of the compounds in the different groups is characterized by the symbols 4+ (very marked), 3+ (marked), 2+ (moderate), 1+ (weak), and 0 (inactive). Even with this simple system the expected error in grading is at least one + symbol. Therefore, the grading of the hydrocarbons considered in this paper (Table 1) has been further simplified by using only four groups: very active,

TABLE 1

Data characterizing chemical reactivity, hydrophobic binding to protein, and carcinogenic activity of a series of polycyclic aromatic hydrocarbons

For explanation and definition of $|K|$, see the text. K_B and m are the binding constant (M^{-1}) and the molar ratio of bound hydrocarbon to protein (M/M), respectively, for the binding of the hydrocarbons to human serum albumin. Binding data marked with an asterisk are experimental values. All other data were calculated from the solubility in water or from the molar refractivity (see the text).

Hydrocarbon	$\|K\|$ (51)	log $\|K\|$	m	$K_B \times 10^{-6}$	log K_B	log $\|K\|$ + log K_B
			M/M	M^{-1}		
Very strongly carcinogenic						
1. 20-Methylcholanthrene	0.9[a]	−0.046	0.030*	5.31*	6.725	6.68
2. 3,4-Benzopyrene	0.71	−0.149	0.064*	4.02*	6.604	6.46
3. 9,10-Dimethyl-1,2-benzanthracene	0.75	−0.125	0.138*	1.37*	6.137	6.01
4. 5,9,10-Trimethyl-1,2-benzanthracene	0.88	−0.056	0.095	2.28	6.358	6.30
5. 5,6,9,10-Tetramethyl-1,2-benzanthracene	1.00	0.000	0.060	3.47	6.540	6.54
6. 5,9-Dimethyl-1,2-benzanthracene	0.75	−0.125	0.149	1.49	6.173	6.05
7. 9,10-Dimethyl-1,2,5,6-dibenzanthracene	0.211	−0.676	0.026	7.59	6.880	6.09
8. 6-Methyl-3,4-benzopyrene	0.57	−0.244	0.117	1.87	6.272	6.03
Strongly carcinogenic						
9. 10-Methyl-1,2-benzanthracene	0.43[a]	−0.367	0.228	1.00	6.000	5.63
10. 9-Methyl-1,2-benzanthracene	0.43[a]	−0.367	0.251	0.93	5.966	5.60
11. 2'-Methyl-1,2,5,6-dibenzanthracene	0.02	−1.699	0.041	4.98	6.697	5.00
12. 5,6-Dimethyl-1,2-benzanthracene	0.088	−1.056	0.149	1.49	6.173	5.12
13. 1,2,5,6-Dibenzanthracene	0.01	−2.000	0.020*	11.23*	7.050	5.05
Weakly carcinogenic						
14. 5-Methyl-1,2-benzanthracene	0.03	−1.523	0.235	0.98	5.991	4.47
15. 8-Methyl-1,2-benzanthracene	0.05	−1.301	0.235	0.98	5.991	4.69
16. 6-Methyl-1,2-benzanthracene	0.04	−1.398	0.235	0.98	5.991	4.59
17. 4-Methyl-1,2,5,6-dibenzanthracene	0.006	−2.222	0.041	4.98	6.697	4.26
18. 7-Methyl-1,2-benzanthracene	0.01	−2.000	0.235	0.98	5.991	4.00
19. 1,2,7,8-Dibenzanthracene	0.38	−0.420	0.016	2.23	6.348	5.93
20. 1,2-Benzanthracene	0.006	−2.222	0.104	2.15	6.332	4.11

Doubtfully carcinogenic						
21. Chrysene	0.001	−3.000	0.036	5.53	6.742	3.74
Not carcinogenic						
22. 1′-Methyl-1,2-benzanthracene	0.001	−3.000	0.235	1.00	6.000	3.00
23. 2,3-Dimethylchrysene	0.0025	−2.602	0.158	1.42	6.152	3.55
24. 1-Methylchrysene	0.0036	−2.444	0.243	0.93	5.969	3.53
25. 2′-Methyl-1,2-benzanthracene	0.003	−2.523	0.235	0.98	5.991	3.47
26. Pyrene	0.005	−2.301	0.445*	0.54*	5.735	3.44
27. Picene	0.0004	−3.398	0.016	4.75	6.677	3.28
28. 3′-Methyl-1,2-benzanthracene	0.005	−2.301	0.235	0.98	5.991	3.69
29. 4′-Methyl-1,2-benzanthracene	0.0007	−3.155	0.235	0.98	5.991	2.84
30. 1,3-Dimethylanthracene	0.002	−2.69ᵇ	0.802	0.31	5.494	2.80
31. 1,2-Benzopyrene	0.0001	−4.000	0.058	3.76	6.575	2.58
32. 9,10-Dimethylanthracene	0.0008	−3.097	0.802	0.31	5.494	2.40
33. Anthracene	0.00017[a]	−3.770	0.219*	0.52*	5.717	1.95
34. 9-Methylanthracene	0.00017	−3.770	1.126	0.21	5.312	1.54

[a] Approximate values (O. Chalvet, personal communication).

active, weakly active, and inactive compounds. The grouping was based on biological data from the literature (28, 36–38, 45–51). For statistical purposes the hydrocarbons in the four groups were arbitrarily assigned the values of 3, 2, 1, and 0 as a measure of their carcinogenic activity. For 9-methyl-1,2-benzanthracene and 10-methyl-1,2-benzanthracene a value of 2.5 was used. The carcinogenicity of chrysene is equivocal (37). Therefore, a value of 0.5 was used rather than 0 or 1. The assignment of 6-methyl-3,4-benzopyrene and 9,10-dimethyl-1,2,5,6-dibenzanthracene to the group of the very active compounds refers to the recent results of Heidelberger *et al.* (48) and Lacassagne *et al.* (50). 9,10-Dimethylanthracene, which appears in the group of inactive compounds in Table 1, is sometimes considered to be slightly active. According to Clayson (37), however, the corresponding experiments were not particularly convincing, so that placement of this hydrocarbon in the group of inactive compounds, which is in agreement with the opinion of other authors (e.g., ref. 51), seems to be justified.

Chemical reactivity (x). For the special purpose of this paper the most suitable quantity for characterizing the chemical reactivity of the hydrocarbons within the framework of the *K-L*-region theory seems to be the $|K|$ value introduced by Chalvet and his co-workers (51, 52). This value is a measure of the ability of a given hydrocarbon to react with protein through the *K*-region, taking into account both the reactivity of the *K*-region and that of the *L*-region, and thus combining the characteristics of both regions in one quantity. A further advantage is that this value is available not only for nonsubstituted but also for substituted hydrocarbons. The $|K|$ value was derived from a very simple model which is based on the following assumptions.

1. The hydrocarbons can react with protein across either the *K*-region or the *L*-region.

2. Either protein (52) or hydrocarbon (51) is present in considerable excess.

3. The complexes formed between hydrocarbon and protein are metabolized and destroyed afterward.

Thus, the following scheme results.

$$\text{Protein} + \text{hydrocarbon} \begin{cases} \xrightarrow{k} C \xrightarrow{k_e} \text{metabolite} \\ \xrightarrow{k'} C' \xrightarrow{k'_e} \text{metabolite}' \end{cases}$$

C and C' are the complexes resulting from the fixation of the hydrocarbon through the *K*-region or the *L*-region, respectively, and k and k' are the rate constants for the formation, and k_e and k'_e the rate constants for the decomposition, of the corresponding complexes. Using steady-state kinetics, it is possible to calculate the concentrations of the complexes C and C' as a function of time.[1] As observed *in vivo*, these quantities pass through a maximum. The $|K|$ value is proportional to the maximum concentration of the complex C, and thus to the amount of hydrocarbon fixed in the "right way" through the *K*-region. Since the rate constants k and k' were obtained from electrophilic *ortho* and *para* localization energies, it was possible to include substituted hydrocarbons in the calculations. It is of interest to note that there exists a certain correlation between the Pullman combined indices for the *K*-region and the electrophilic *ortho* localization energy (51): if the Pullman index is smaller than 3.31β, the value for the electrophilic *ortho* localization energy will be less than 3.64β (but see also ref. 53).

As is to be expected and as can be seen from Table 1, there is a fairly good correlation between $|K|$ and carcinogenic activity, the correlation coefficient being 0.83; however, the relationship between the $|K|$ values and the activity is not linear but exponential. Therefore, the quantity $\log |K|$ is used to characterize the chemical reactivity of the hydrocarbons in the correlations with carcinogenic activity: $x = \log |K|$. The correlation coefficient r_{xy} for the correlation of carcinogenic activity (y) with $\log |K|$ (x)

[1] This calculation should also be valid if the reaction through the *K*-region and through the *L*-region occurs with different proteins.

is equal to 0.90, and the corresponding rank correlation coefficient amounts to 0.92.

Hydrophobic binding to protein (z). A reasonable assumption as to how physical (hydrophobic) binding of the hydrocarbons to protein may influence the process of chemical carcinogenesis could be that physical binding is a necessary prerequisite for the critical chemical reaction to occur. If this is accepted, then it could be either the total amount of hydrocarbon physically bound or the strength of binding which is related to carcinogenesis. A glance at Table 1 shows that the first possibility seems rather unlikely, if the molar ratio m of bound hydrocarbon to protein for the binding of the hydrocarbons to human serum albumin is considered. It is immediately evident that there is no association between the m values and carcinogenic activity. Typical inactive hydrocarbons like phenanthrene, anthracene, or pyrene show the highest values of m, whereas extremely potent compounds like 3,4-benzopyrene or 20-methylcholanthrene have very low m values. The second possibility implies that the change in standard free energy on physical binding is an important factor in chemical carcinogenesis. This being the case, there should exist a correlation between the logarithms of the binding constants (log K_B) and carcinogenic activity. Correlation analysis yields a correlation coefficient of 0.48. The corresponding rank correlation coefficient amounts to 0.52. Both coefficients are statistically significant, with $p < 0.01$, thus indicating an association between logarithms of the binding constants and carcinogenic activity. Hence, we have defined: $z = \log K_B$.

As already pointed out, the binding data used in this paper refer to our binding studies with human serum albumin as a model (23–26). The validity of this model for the situation *in vivo* will be discussed below. The figures in Table 1 marked with an asterisk are experimental values. All other binding data were calculated either from the solubility in water if solubility data were available (data from ref. 54) or from the molar refractivity (data from ref. 55) with the help of the equations presented in ref. 26.[2] These calculations are based on the assumption that protein binding may be treated as a more or less complete reversal of the solution process in water (26) (see also ref. 56). Though for crystalline hydrocarbons the state of molecules bound to protein is not strictly identical with the state of the molecules in the pure hydrocarbon, there is good experimental evidence that this concept provides at least a very good approximation for such compounds. In the case of the polycyclic aromatic hydrocarbons, for example, an excellent correlation exists between the free energy of binding to human serum albumin and the free energy for the solution process in water (26), the correlation coefficient being −0.998.[3] Corresponding correlations have also been presented for the binding of other series of solid aromatic compounds to bovine serum albumin (17, 58), as well as to enzymes (59, 60).

The binding constants for the polycyclic aromatic hydrocarbons can also be calculated from partition coefficients (26). Partition coefficients for such compounds, most of them estimated theoretically, have been presented by Hansch and Fujita (61). In general, the agreement of binding constants calculated from solubilities in water and from these partition coefficients is quite satisfactory. For some methyl-substituted hydrocarbons, however, binding constants obtained from solubilities are lower than those obtained from partition coefficients. Fortunately, there is agreement in two important points: binding constants for isomeric methyl derivatives are always about equal, and with increasing numbers of methyl substituents the logarithms of the

[2] Since hydrocarbons with more than five fused rings had not yet been included in our protein binding studies, it is not certain whether these equations are valid for such hydrocarbons. Therefore, hydrocarbons with more than five fused rings could not be treated in the present investigation.

[3] A similar relationship exists for the solubilization of polycyclic aromatic hydrocarbons in potassium laurate micelles, as follows from the data of Klevens (57).

binding constants increase steadily. Thus, for the problem the present paper is concerned with, the difference in the binding data resulting from solubilities and partition coefficients for some hydrocarbons should not be critical. Nevertheless, as a control, it seems desirable to repeat the statistical tests, using binding constants calculated from partition coefficients for the substituted hydrocarbons in question. This will be done with the ranking technique of Kendall (34), which permits calculation of the partial and multiple rank correlation coefficients. If the binding constants for the substituted hydrocarbons are calculated from partition coefficients, the following rank for log K_B is obtained (same numbering of the hydrocarbons as in Table 1).

$$7 > 11 = 17 > 5 > 13 = 4 > 1 = 8 > 3 = 6 = 12 > 21 > 27 > 23 > 2 > 31 > 9 = 10 = 14 = 15 = 16 = 18 = 22 = 25 = 28 = 29 > 19 > 24 > 20 > 26 > 30 = 32 > 34 > 33$$

The last point to be discussed here is the validity of human serum albumin as a model. There are some results available from the literature (62–65) which indicate that polycyclic hydrocarbons indeed interact physically with cellular proteins *in vivo*. These proteins are, of course, different from human serum albumin, but this fact presents no serious problem. It follows directly from the equations presented previously (26) that the constants for the binding of hydrocarbons to a protein (I) are related to the corresponding constants for the binding to another protein (II) according to Eq. 1:

$$(\log K_B)_{II} = a(\log K_B)_I + b \qquad (1)$$

This equation is valid as long as only unspecific hydrophobic forces are present and no steric hindrance of the binding occurs. Assuming this to be true, the relative order of the binding constants is the same for all proteins. Therefore, human serum albumin seems to be acceptable as a model.

Partial and Multiple Correlations and Regressions between Carcinogenic Activity (y), Chemical Reactivity (x), and Hydrophobic Binding to Protein (z) of the Hydrocarbons

It was shown in the preceding section that there exists a close correlation between carcinogenic activity and the logarithms of the $|K|$ values. Furthermore, a loose association between carcinogenic activity and the logarithms of the binding constants is also highly probable. If the partial and the multiple correlation coefficients are calculated, this picture is fully confirmed. All coefficients are summarized in Table 2. Since both the partial coefficient $r_{zy \cdot x}$ and the multiple coefficient $r_{y \cdot xz}$ are statistically significant, with $p < 0.01$, and since the multiple coefficient is greater than the partial coefficients, it is to be concluded that hydrophobic interactions between hydrocarbons and proteins are indeed involved in the process of chemical carcinogenesis. From the values of $r_{xy \cdot z}$, $r_{zy \cdot x}$, and $r_{y \cdot xz}$ it is evident, however, that the role of these interactions is only of secondary importance as compared with the chemical reactivity of the hydrocarbons. Equation 2 was derived by the method of least squares from the data presented in Table 1.

TABLE 2

Simple, partial, and multiple correlation coefficients for the correlations between three variables characterizing carcinogenic activity (y), chemical reactivity (x), and hydrophobic binding to protein (z) of the 34 hydrocarbons listed in Table 1

Simple correlations	Simple rank correlations[a]	Partial correlations	Multiple correlation
$r_{xy} = 0.90$	$R_{xy} = 0.92$	$r_{xy \cdot z} = 0.90$	$r_{y \cdot xz} = 0.93$
$r_{zy} = 0.48$	$R_{zy} = 0.52$	$r_{zy \cdot x} = 0.49$	
$r_{xz} = 0.31$	$R_{xz} = 0.36$		

[a] According to Spearman.

$$y = -1.79 + 0.75x + 0.71z \quad (2)$$

An analysis of variance shows that the z term in Eq. 2 is quite significant, with $p < 0.01$. For all practical purposes the two constants 0.75 and 0.71 may be considered approximately identical. It then follows from Eq. 2 that the carcinogenic activity should be proportional to the sum of log $|K| + \log K_B$. This sum is presented in the last column of Table 1. As can be seen, there is a good correlation of this sum with carcinogenic activity. All hydrocarbons in the group of the very active compounds have values greater than 6, the values for the compounds in the second group are between 5 and 5.63, and the values in the third group are between 4 and 4.69, the only exception being 1,2,7,8-dibenzanthracene. All inactive compounds have values smaller than 3.70. Thus, the sum log $|K| + \log K_B$ permits an exact grouping of the hydrocarbons according to their carcinogenic activity. This is not possible with the help of $|K|$ or log $|K|$ alone. For example, 9,10-dimethyl-1,2,5,6-dibenzanthracene would seem to belong to the second group of hydrocarbons rather than to the first group according to its relatively low $|K|$ value of 0.211, and correct assignment of the compounds belonging to the second and third groups in Table 1 would not be possible at all. Clearly, the simultaneous consideration of both chemical reactivity and hydrophobic binding to protein provides better insight into the problem of carcinogenic activity than the consideration of chemical reactivity alone. Essentially the same conclusions are reached if the statistical analysis is repeated with the rank of log K_B which was obtained using partition coefficients for the calculation of the binding constants for substituted hydrocarbons (see above). The simple, partial, and multiple rank correlation coefficients are summarized in Table 3.[4] Since both the partial rank correlation coefficient $R'_{zy \cdot x}$ and the multiple coefficient $R'_{y \cdot xz}$ are statistically significant, with $p < 0.01$, the relationship between hydrophobic binding to protein and carcinogenic activity can be considered as being proven in this case also.

[4] The lower values of the coefficients in Table 3 as compared with the coefficients in Table 2 are due to the special characteristics of the rank correlation coefficient according to Kendall.

TABLE 3

Simple, partial, and multiple rank correlation coefficients according to Kendall for the correlations between three variables characterizing carcinogenic activity (y), chemical reactivity (x), and hydrophobic binding to protein (z) of the 34 hydrocarbons listed in Table 1

The rank for z was obtained using partition coefficients to calculate the binding constants for the substituted hydrocarbons (see the text).

Simple rank correlations	Partial rank correlations	Multiple rank correlation
$R'_{xy} = 0.82$	$R'_{xy \cdot z} = 0.78$	$R'_{y \cdot xz} = 0.84$
$R'_{zy} = 0.52$	$R'_{zy \cdot x} = 0.37$	
$R'_{zx} = 0.40$		

Figure 1 shows a plot of the sum log $|K| + \log K_B$ with respect to carcinogenic activity. Though this plot again demonstrates the close relationship between these two quantities, it is also evident that direct proportionality is limited to the range of active compounds. It is obvious from Table 1 that the division of the hydrocarbons into active and inactive classes is possible with the help of the simple $|K|$ values alone, if the value for 1,2-benzanthracene is taken as a threshold. The advantage of the sum log $|K| + \log K_B$ over the simple $|K|$ or log $|K|$ values is only that it permits the correct grading of the hydrocarbons *within* the group of active compounds. Therefore, it was of interest to repeat the statistical analysis for the 21 active compounds alone. The correlation coefficients are summarized in Table 4. The conclusions reached from this table are essentially the same as from Table 2, since the levels of significance for $r_{zy \cdot x}$, $r_{zy \cdot x}$, and $r_{y \cdot xz}$ are the same in both cases. Note the pronounced increase in the partial correlation coefficients as compared with the simple coefficients, especially for the correlation between log K_B and the carcino-

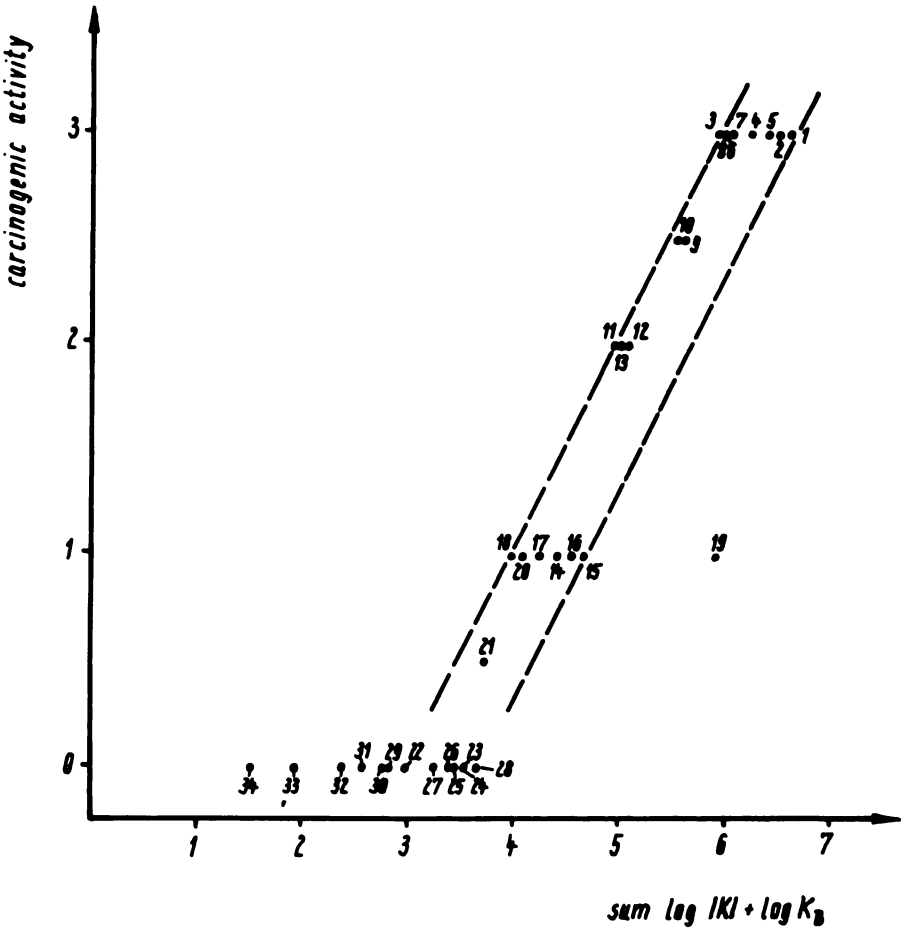

FIG. 1. *Relationship between the sum log $|K|$ + log K_B and the carcinogenic activity*
The numbering of the hydrocarbons is the same as in Table 1. For the explanation, see the text.

genic activity. The method of least squares yields Eq. 3 for the 21 active compounds.

$$y = -2.93 + 0.91x + 0.92z \quad (3)$$

An analysis of variance again demonstrates the significance of the z term ($p < 0.01$). The two constants 0.91 and 0.92 are practically identical, so that the use of the sum log $|K| + \log K_B$ as an index of carcinogenic activity is substantiated.

Some inactive hydrocarbons (phenan-

TABLE 4
Simple, partial, and multiple correlation coefficients for the correlations between three variables characterizing carcinogenic activity (y), chemical reactivity (x), and hydrophobic binding to protein (z) of the 21 active compounds listed in Table 1

Simple correlations	Simple rank correlations[a]	Partial correlations	Multiple correlation
$r_{xy} = 0.82$	$R_{xy} = 0.85$	$r_{xy \cdot z} = 0.88$	$r_{y \cdot xz} = 0.89$
$r_{zy} = 0.16$	$R_{zy} = 0.17$	$r_{zy \cdot x} = 0.59$	
$r_{xz} = -0.20$	$R_{xz} = -0.07$		

[a] According to Spearman.

threne, pentaphene, and 1,2,3,4,-dibenzanthracene) listed by Chalvet (51) have $|K|$ values equal to zero. Since the logarithm of zero is meaningless, these hydrocarbons could not be included in the present investigation. In reality, however, the $|K|$ values of these hydrocarbons would only be too small to be distinguishable from zero with the model applied by Chalvet. It may be deduced from his table that the logarithms of these $|K|$ values must be smaller than -5. This being the case, the values for the sum $\log |K| + \log K_B$ for the hydrocarbons mentioned above would be in the right order of magnitude for inactive compounds.

As already pointed out, binding constants for hydrocarbons with more than five fused rings are not available at present. Therefore, additional hydrocarbons (3,4,8,9-dibenzopyrene, 3,4,9,10-dibenzopyrene, 1,2,7,8-dibenznaphthacene, and anthanthrene) presented in the paper of Chalvet had to be excluded. Three dimethyl-1,2-benzanthracenes with one of the methyl groups in the angular ring (1′, 10-, 3′,6-, and 3′,7-dimethyl-1,2-benzanthracene), also listed by Chalvet, provide a special case. Hydrocarbons of this type proved to be a pitfall in any attempt so far made to correlate structure or certain properties of the hydrocarbons with carcinogenicity. Usually 1,2-benzanthracene derivatives with one methyl group in the angular ring are considered completely inactive. There are, however, some recent reports which indicate that such compounds could have very weak activity. Graffi and Bielka (38) consider 1′,10-dimethyl-1,2-benzanthracene to be very slightly active, and according to Heidelberger *et al.* (48) the same is true for 2′,6-dimethyl-1,2-benzanthracene. Further examples of possibly weak activity among such compounds are 4′,10-dimethyl-1,2-benzanthracene and 4′,9,10-trimethyl-1,2-benzanthracene (see ref. 66). Thus, the inactivity of such compounds must be considered with some caution. Furthermore, 1′,10-dimethyl-1,2-benzanthracene, as well as 3′,6- and 3′,7-dimethyl-1,2-benzanthracene, shows steric peculiarities which make the calculation of binding constants from the molar refractivity (solubility data are not available for these hydrocarbons) somewhat difficult: the former hydrocarbon is not planar, and the latter two have molecular dimensions distinctly greater than for other dimethyl-1,2-benzanthracenes.[5] For these reasons these hydrocarbons were also not included in the present investigation. This does not, however, affect the principal result of this paper.

Comparison with the Approach of Hansch and Fujita

It has been shown in the preceding section that hydrophobic binding of the hydrocarbons to protein is of importance for the process of chemical carcinogenesis, the carcinogenic activity being linearly related to $\log K_B$. Another interesting approach relating hydrophobic properties of the hydrocarbons with carcinogenesis is that of Hansch and Fujita (61). Applying their brilliant *pi-sigma* treatment to substituted 1,2-benzanthracenes, benzacridines, and benzphenanthrenes, these authors derived the following equation:

$$\log A = -0.14(\log P - 5)^2 + 0.32(\log P - 5) + 28.07\epsilon - 35.26 \quad (4)$$

In Eq. 4, A[6] is the carcinogenic activity on an arbitrary scale, P is the partition coefficient in the system octanol–water (calculated from increments), and ϵ is the total charge on the K-region according to Pullman.

Let us first consider the role of $\log P$ in Eq. 4 and its relationship to $\log K_B$ in the

[5] N. P. Buu-Hoi, personal communication.

[6] In Eq. 4 $\log A$ is considered, whereas the equations presented in the present paper contain the activity on a linear scale. However, because of the rather crude system of grading which had to be adopted in both papers because of the uncertainties inherent in the biological results, it does not matter much whether A or $\log A$ is used in the statistical analyses. For example, Eq. 3 of this paper may be substituted by the following equation without much change.

$$\log y = 0.23x + 0.22z - 0.93 \qquad (r_{y.xz} = 0.86)$$

Thus, this difference is not important and needs no special discussion.

equations presented above. According to the model of Hansch and Fujita, which has become so well known that it need not be considered here in detail, the relationship between activity and partition coefficients is due to the movement of the hydrocarbons to the site of action. These authors consider this movement to be a random walk process which depends on the lipohydrophilic character of the molecules concerned. They assume that there is an ideal value of log P such that any deviation from this value will result in a slower rate of movement to the site of action and, consequently, in a decrease in biological activity. Mathematically this results in a parabolic relationship between log A and log P, as in Eq. 4. Thus Eq. 4 means that the lower hydrocarbons are not lipophilic enough and hydrocarbons beyond a certain size are too lipophilic to achieve maximum concentration at the site of action and, hence, to possess maximum activity, the ideal value of log P being about 6.1. However, experimental data on the uptake of hydrocarbons by mouse skin after painting with a 1% solution in benzene (plus 1% mineral oil) presented by Bock and Burnham (67, 68) seem not to favor this concept. If a true equilibrium is established, the uptake of hydrocarbons by skin may be considered a special case of a partition equilibrium with the tissue as "aqueous" phase. It may then be predicted from the equations presented previously (26) (see also ref. 69) that a plot of the logarithm of the concentration of hydrocarbon in skin with respect to the negative logarithm of the solubility in water should yield a straight line with negative slope. As shown in Fig. 2, this is indeed the case, and the agreement of the rather difficult-to-determine biological data with this straight line may be considered quite satisfactory. The negative logarithm of the solubility in water, being a measure of hydrophobicity, is related to the logarithms of partition coefficients within homologous series of hydrophobic substances (26, 69).[7] Thus, it follows from Fig. 2 that the concentration of hydrocarbon in skin decreases with increasing partition coefficients or increasing hydrophobicity, respectively. According to the model of Hansch and Fujita, however, a parabolic relationship with the maximum near 1,2-benzanthracene should have been expected rather than a straight line, since the lower hydrocarbons considered in Fig. 2 have values of log P well below the ideal value of 6.1. Though the concentration of hydrocarbon in the whole tissue is not necessarily identical with the concentration of hydrocarbon at the site of action within the cell, Fig. 2 strongly suggests that the squared term in Eq. 4 is of doubtful significance. This term is indeed a troublesome one, since it leads to completely wrong predictions of the carcinogenic activity of higher condensed hydrocarbons. According to Eq. 4, 9,10-dimethyl-1,2,5,6-dibenzanthracene should be only very slightly active, and hydrocarbons with six or more fused rings should be inactive, since these hydrocarbons are supposed to be much too lipophilic. In reality, however, 9,10-dimethyl-1,2,5,6-dibenzanthracene is a very strong carcinogen (48), which fact is correctly predicted by the sum log $|K| + \log K_B$ (see Table 1), and hydrocarbons as big as phenanthro(2′,3′,3,4)pyrene (6), 1,2,4,5,8,9-tribenzopyrene (6, 70), and peropyrene (71) have been proved to possess definite carcinogenic activity. If, on the other hand, the squared term in Eq. 4 is dropped, the situation is not improved, since then the higher alkyl derivatives of 1,2-benzanthracene and 20-methylcholanthrene would

[7] Instead of the negative logarithm of solubility, log P values could also have been used, in principle, in the plot presented in Fig. 2. Solubility data have been preferred for three reasons: (a) log P values for 3,4-benzopyrene and methyl-3,4-benzopyrene are lacking, (b) the solubilities are determined experimentally (54) whereas the log P values are only theoretical estimates, and (c) the log P values calculated by Hansch and Fujita might be somewhat too high for some substituted hydrocarbons (see ref. 26). Together with the polycyclics, three benzacridines were also included in the studies of Bock and Burnham. These hydrocarbons cannot be handled in the same plot with the polycyclics since they do not belong to the same homologous series.

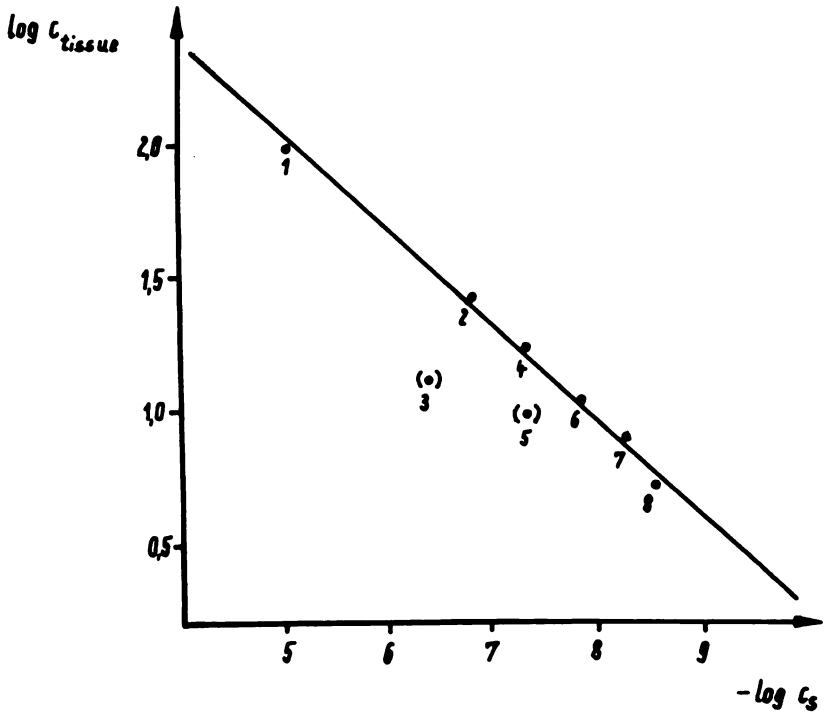

FIG. 2. *Relationship between the logarithm of the concentration of hydrocarbon in mouse skin after painting with a 1% solution in benzene (+1% mineral oil) (log c_{tissue}) and the negative logarithm of the solubility in water (−log c_S) for several polycyclic aromatic hydrocarbons*

Biological data are taken from Bock and Burnham (67), and solubility data from Davis *et al.* (54) (for 10-propyl-1,2-benzanthracene interpolated according to ref. 24). *1* = phenanthrene, *2* = 9,10-dimethyl-1,2-benzanthracene, *3* = anthracene, *4* = 10-propyl-1,2-benzanthracene, *5* = 1,2-benzanthracene, *6* = 3,4-benzopyrene, *7* = 20-methylcholanthrene, *8* = 6-methyl-3,4-benzopyrene. For explanation, see the text.

appear to have increasing activity with increasing length of the carbon chain. Thus, one is led to the conclusion that the protein-binding concept outlined in the present paper is probably more adequate to rationalize the influence of hydrophobicity on chemical carcinogenesis than the kinetic model of Hansch and Fujita. In fact, it is not necessary to invoke this model to account for the relationship found between activity and log P, since the protein-binding concept can explain this relationship equally well; it can also provide a simple explanation for the existence of the squared term in Eq. 4. If physical binding to protein is considered the real mechanism by which the hydrophobic character of the hydrocarbons influences carcinogenesis, the relationship found between activity and log P is simply ascribable to the fact that the logarithms of partition coefficients are linearly related to the logarithms of binding constants (26, 69). In other words, the log P term in Eq. 4 is actually a log K_B term, log P being only a measure of log K_B. In the case of the higher alkyl 1,2-benzanthracenes considered by Hansch and Fujita, however, log P cannot be used as a measure of log K_B, since the protein binding of these compounds is expected to be sterically hindered (see DISCUSSION). For these compounds we have, with increasing length of the carbon chain, (a) a strong decrease in activity at about constant chemical reactivity, (b) decreasing values of log K_B as a result of steric hindrance, and (c) increasing values of log P. The decrease in log K_B is sufficient to explain the decrease in activity on the basis of a linear relationship according to the equa-

tions presented in this paper (see DISCUSSION). If, however, log P instead of log K_B is considered, a squared term according to Eq. 4 becomes necessary to account for the decrease in activity with increasing values of log P. Thus, it follows that the squared term in Eq. 4 is probably due mainly to the inadequacy of log P as a measure of log K_B in the case of the higher alkyl 1,2-benzanthracenes. In the opinion of the present author, this term most likely has no real physical significance, and it is not needed if the partition coefficients are replaced by binding constants.[8]

In addition to the wrong conclusions concerning the carcinogenic activity of higher condensed hydrocarbons already mentioned, carcinogenic activity in general is only poorly predicted by Eq. 4 as compared to the sum log $|K|$ + log K_B presented in this paper. This follows from Fig. 3, which shows the correlation between observed and calculated activities for the active hydrocarbons considered in the paper of Hansch and Fujita. A rather irregular scattering of points is obtained (compare with Fig. 1), and it is immediately evident that correct grading of hydrocarbons according to their carcinogenic activity is not possible with the help of Eq. 4. Furthermore, four out of 12 inactive compounds (not included in Fig. 3) would appear to be active according to the log A values calculated from this equation. These inaccuracies in grading can also be explained on the basis of the protein-binding concept. It is to be expected that somewhat different values for the constants in the linear equation relating log K_B to log P are required for the different classes of hydrocarbons considered in the paper of Hansch and Fujita.[8] This is not taken into account by the simple log P values and may well be a reason for the scattering of points in Fig. 3.

There is still another important point to be discussed in this section concerning the role of chemical reactivity. In the present paper the roles of both the K-region and the L-region are emphasized, physical binding to protein being considered a factor of secondary importance as compared with chemical reactivity. Hansch and Fujita, on the other hand, consider the role of the lipohydrophilic character of the hydrocarbons more important and conclude that "it is not necessary to invoke the concepts of L and K regions." This conclusion is based mainly on the assumption that hydrocarbons with six or more fused rings are inactive. This, however, is not true, as already discussed. Furthermore, it has been pointed out by the Pullmans (27) that the carcinogenic activity of substituted and azo derivatives of polycyclic aromatic hydrocarbons often depends mainly on the reactivity of the K-region alone. Conclusions on the general importance of the L-region cannot, therefore, safely be drawn from the sample of hydrocarbons considered by Hansch and Fujita. Although, according to the statement of the Pullmans, the influence of the L-region may be neglected in a first approximation for many of these hydrocarbons, the use of the simple data of the total charge on the K-region in deriving Eq. 4 is very likely to be another reason for the shortcomings of this equation in predicting carcinogenic activity.

It is to the credit of Hansch and Fujita that they were the first to show, by a de-

[8] The most convenient way to substantiate this conclusion for the hydrocarbons considered by Hansch and Fujita would, of course, be a statistical analysis using binding constants instead of partition coefficients. However, binding constants for the benzphenanthrenes and for the benzacridines are not available and cannot be calculated from the equations derived previously (26) for planar polycyclic aromatic hydrocarbons. The nonplanarity of the ring system in the benzphenanthrenes, as well as the presence and position of the nitrogen in the benzacridines, is expected to influence protein binding, so that other sets of constants for these equations are required for these compounds (see also ref. 69). Therefore, a statistical analysis cannot be made for the present. However, since no squared term is needed in the equations presented in this paper, though some of the hydrocarbons in Table 1 have log P values well above the ideal value quoted by Hansch and Fujita, it seems rather safe to conclude that the same is true for the sample of hydrocarbons considered by these authors.

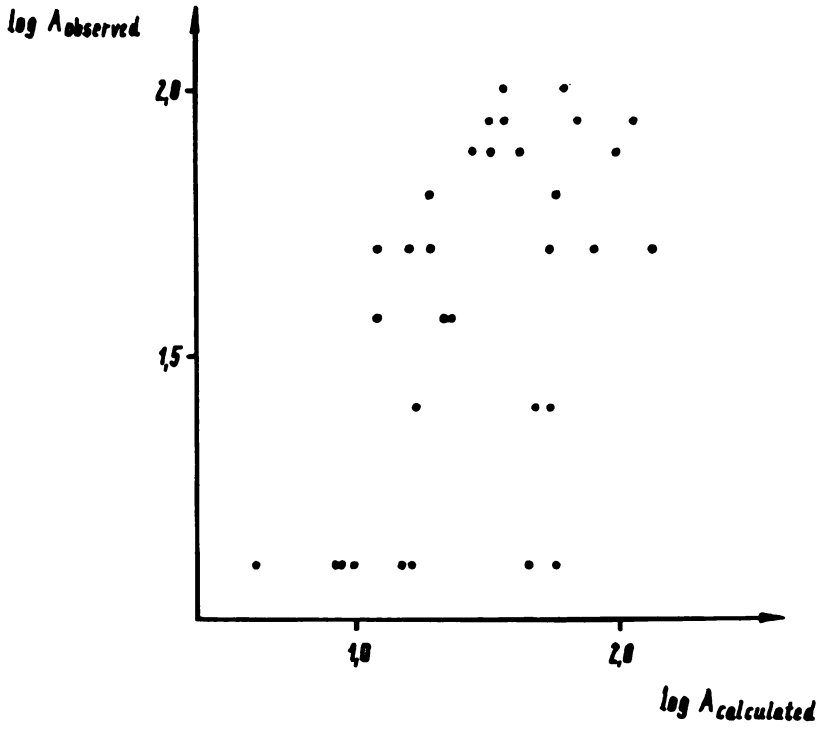

FIG. 3. *Relationship between the logarithm of the carcinogenic activity observed (log $A_{observed}$) and log A calculated from the equation presented by Hansch and Fujita (log $A_{calculated}$) for the active hydrocarbons consid red by these authors*

All data are taken from Table VII of Hansch and Fujita (61). Note the scattering of points.

tailed analysis, that hydrophobicity is involved in carcinogenesis. The kinetic model of these authors has proved to be extremely useful in structure-activity analysis for many series of biologically active substances (e.g., ref. 72). Hansch *et al.* realized in a later paper (72) that, in principle, relationships between hydrophobicity and biological activity may also be due to protein binding, but they emphasized at the same time that they still favor the kinetic model. In the special case of polycyclic aromatic hydrocarbons, however, it is the feeling of the present author that the protein-binding concept as outlined above is preferable. This concept, in combination with the *K-L*-region theory, not only has the advantage of allowing correct grading of the hydrocarbons according to their carcinogenic activity, but can also offer a simple explanation for the interference among hydrocarbons in skin carcinogenesis (see below), which the kinetic model cannot.

DISCUSSION

The principal result of this paper is in good agreement with both the protein deletion hypothesis and the *K-L*-region theory of chemical carcinogenesis, and it may be summarized as follows. Hydrophobic interactions of hydrocarbons with proteins do influence the process of chemical carcinogenesis, but their role is only of secondary importance as compared with the chemical reactivity of the hydrocarbons. With increasing strength of these interactions, carcinogenic activity increases. Thus, these interactions are necessary and favorable for the process of chemical carcinogenesis and somehow seem to facilitate the reaction which leads to the malignant change in the cell. Any alteration of a given hydrocarbon which, at constant

chemical reactivity, leads to a decrease in binding strength should therefore result in a decrease in carcinogenic activity. This conclusion offers a simple and straightforward explanation for the drop in activity which is usually observed for substituents with polar characteristics, since the introduction of such substituents unfavorable for hydrophobic interactions lowers the binding constants. The same line of reasoning may be applied to explain the decrease in activity with increasing length of alkyl substituents in alkyl-1,2-benzanthracenes and 20-alkylcholanthrenes. It was shown in a preceding paper (26) [see also Sahyun (17)] that the hydrocarbons bound to protein are most likely flatly adsorbed to a hydrophobic region on the surface of the protein molecule. The presence of bulky substituents such as big alkyl groups in the hydrocarbon molecules would sterically hinder the binding process (see also ref. 73) and hence would lead to a decrease in the binding constants. A similar situation is to be expected for very large hydrocarbons. It follows from the equations presented elsewhere (26, 69) that binding constants increase with increasing molecular size. However, if an isochemical series ascends sufficiently far, a member will be reached which is too big for the binding region in the protein molecule (see refs. 26 and 69). Beyond this member each further increase in molecular size will result in a decrease in the binding constants and, therefore, in a decrease of carcinogenic activity at a given level of chemical reactivity. Molecular structure may be quite critical here, since it is probably the greatest length and the greatest width of the hydrocarbon molecules rather than the total number of aromatic rings or substituents which are important. Without detailed knowledge of the protein(s) of the cell involved in carcinogenesis, valid conclusions on size and shape of the binding region cannot, of course, be drawn, but it seems that this region is rather large, since hydrocarbons with more than six fused rings have been shown to possess definite carcinogenic activity (6, 70, 71).

The relationship between the logarithms of the binding constants and carcinogenic activity indicates that the influence of hydrophobic binding on carcinogenesis is probably related to the free energy of binding. One reasonable suggestion might be that binding changes the conformation of a receptor protein in such a manner that the subsequent chemical reaction is facilitated and that this conformational change depends on the free energy of physical binding. Such a relationship between binding energy and conformational change of the binding protein exists, for example, in the case of the binding of some anesthetics to globular proteins, as can be deduced from the data of Balasubramanian and Wetlaufer (74). Another possibility is that the energy of binding provides part of the energy of activation for the critical chemical reaction. Further studies are certainly needed to clarify this point.

It has been shown by several workers (75–83) that inactive and weakly active hydrocarbons have an inhibitory effect on the carcinogenic potency of strongly active compounds. Competition for a cellular receptor is the usual explanation offered for such inhibition. In more recent investigations (e.g., ref. 82) it has been assumed that this competition occurs in a chemical reaction with a protein of the cell. However, it has been shown by Abell and Heidelberger (84) that active and inactive hydrocarbons react with different proteins. Thus, competition in a chemical reaction can provide no straightforward explanation for the interference among hydrocarbons in carcinogenesis. In the opinion of the present author, hydrophobic binding is involved in this competition, rather than a chemical reaction. Although the inactive or weakly active compounds might be unable or only barely capable of reacting chemically with the specific receptor protein for the active hydrocarbons, these compounds could well compete with the strong carcinogens for the same hydrophobic binding site. Assuming that physical binding of the active hydrocarbons to the receptor protein is a necessary prerequisite for the critical chemical reaction to occur, this would clearly result in a decrease in activity.

Therefore it seems that the concept presented in this paper may also lead to a better understanding of the inhibitory action of inactive and weakly active hydrocarbons.

The results presented here have a most direct bearing within the framework of the protein deletion hypothesis. They may also, however, be consistent with a reaction of hydrocarbons with DNA. In recent years much interest has focused on the possible role of DNA in chemical carcinogenesis (e.g., refs. 85–88). Like proteins, DNA can react chemically (85–87) as well as physically (88–94; but see also ref. 95) with hydrocarbons. Though the role of physical interactions between hydrocarbons and DNA is not yet known, these interactions are at least partly governed by the same forces as the interactions with proteins. Therefore, similarities in the binding of hydrocarbons to DNA and to proteins are to be expected. On the other hand, it must be stressed that the situation with DNA is more complicated than with protein, since steric peculiarities of the hydrocarbons should exert a much greater influence in this case. Furthermore, the formation of charge transfer complexes may also be of importance (96).

An interesting new approach to rationalizing structure-activity relationships of several diverse classes of carcinogens has suggested recently by Dipple *et al.* (97), who proposed that the ultimate carcinogens which react with the essential cellular receptor may be carbonium ions generated metabolically. Since the site of metabolic generation is supposed to be distant from the receptor site, the stability of these carbonium ions is considered the most important factor determining carcinogenic potency. For polycyclic aromatic hydrocarbons this assumption is supported by a positive correlation between delocalization coefficients and carcinogenic activity. Since the rate of production of carbonium ions and possibly their stability, as well as complex formation with the cellular receptor, are expected to be influenced by hydrophobic interactions, the concept of hydrophobic binding as a secondary factor in carcinogenesis, as outlined in the present paper, is not inconsistent with this theory. Furthermore, the correlation between delocalization coefficients and carcinogenic activity gives a prediction of carcinogenic potencies very similar to those of the *K-L*-region theory according to the combined indices of the Pullmans (27).

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