BIOLOGIC SIGNIFICANCE OF THE STRUCTURE OF HYDROCARBONS

I. Chain Structure

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INTRODUCTION

In 1936–38 Ettisch and Gomes da Costa (3–7) studied the biologic effect of non-aqueous solutions as compared with their aqueous counterparts. The solvents used were vegetable oils and nujol. Only the action of these oils was studied by Ettisch and da Costa (8). Later *n*-decane, dioxane, and triolein were explored by Ettisch (9). It was found that the oils could be used as biologic media; dioxane kills the organism almost immediately.

Ettisch (10, 11) showed that "probability statistics" in the form evolved by von Mises (14, 15) can solve some important problems that descriptive statistics, usually employed, must leave open. That method was used throughout our work.

We turn now to our experiences with short hydrocarbon chain molecules.

EXPERIMENTAL PART

The animals, *Ascaris suis*, female, were brought to the laboratory in thermos bottles containing Rhode-Saito electrolyte combination (abbreviated Rh-S) at 38°C. and were washed there with fresh Rh-S at 38°C. The 4 cm. of the cephalic segment were cut from the trunk, dried carefully with filter paper, shaken rapidly in several samples of the respective hydrocarbons, and finally mounted on a simple lever (Fig. 1), as Rebelo and his coworkers (16) had done. This contrivance was then immersed in a specially designed vessel containing the particular liquid to be tested. The contractions of the segment were recorded by the lever on blackened paper mounted on a rotating drum. The vessel with the liquid and the cephalic segment was then placed in a thermostat of 38.0 ± 0.5°C. About 6 minutes elapsed from the moment of the selection of the *Ascaris* until regular recording began.

This method is an "understatement" method. The lever cannot indicate contractions where the amplitudes are very large. All the registered movements are from medium-sized downward only. Furthermore, only the movements about one axis of the lever can be registered, that is, about an axis in the direction of the radius of the rotating drum or about axes which have a component in that direction.

The substances employed were *n*-pentane, *n*-hexane, and *n*-heptane. They were twice fractionally distilled. Only the fraction closest to the figures in the tables of temperature and pressure was then distilled for a third time. All this was carried out

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in the dark, and after distillation the substances were protected from the light. The substances were dried with properly prepared metallic sodium or dehydrated $CuSO_4$. The purer the chain hydrocarbon and the more thoroughly it was dried, the longer was the mean lifetime and the better the contractions. The density of the liquids was also measured at 28°C.; making due allowance for reasonable error, it should be that found in Landolt Börnstein's tables. The *Ascaris* results were finally reproduced so exactly that these experiments could be used to test the purity of the respective hydrocarbons.

On reading the curves an allowance of ±5 minutes was made. In other words, the readings were corrected in all cases within a range of 10 minutes.

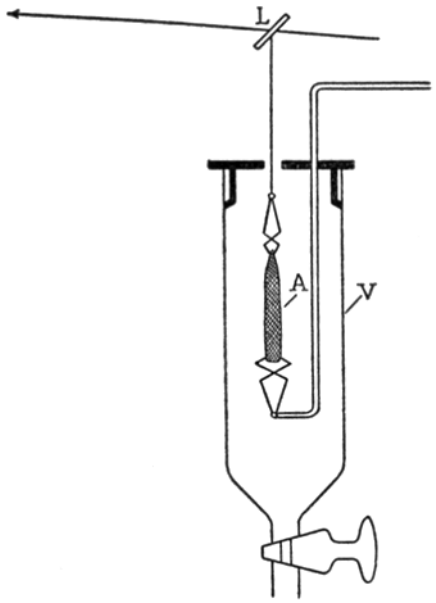

FIG. 1. *A*, *Ascaris* cephalic segment; *L*, lever; *V*, vessel.

n-Pentane.—The expectancy value is $\bar{\tau} = 183 \pm 12$ minutes. That is, if a sufficient[1] number of experiments are carried out, *Ascaris suis*, female, will contract in *n*-pentane so that, on the average, the observed lifetime will be between 171 and 195 minutes. The mortality, *i.e.* the part sum or death probability up to the expectancy value—up to 183 minutes—is $P(\bar{\tau}) = 0.63$. That is to say, if we conduct a sufficient number of this type of experiments in *n*-pentane, we observe that on the average, 63 out of 100 *Ascaris* will be dead after 183 ± 12 minutes. The thermostats maintained a constant temperature of 38 ± 0.5°C. The boiling point of *n*-pentane is close to 36°C. Fig. 2 is a representation of the contractions of the *Ascaris* over a period of more than 2 hours in a boiling liquid.

[1] Theoretically, an infinitely great number of experiments.

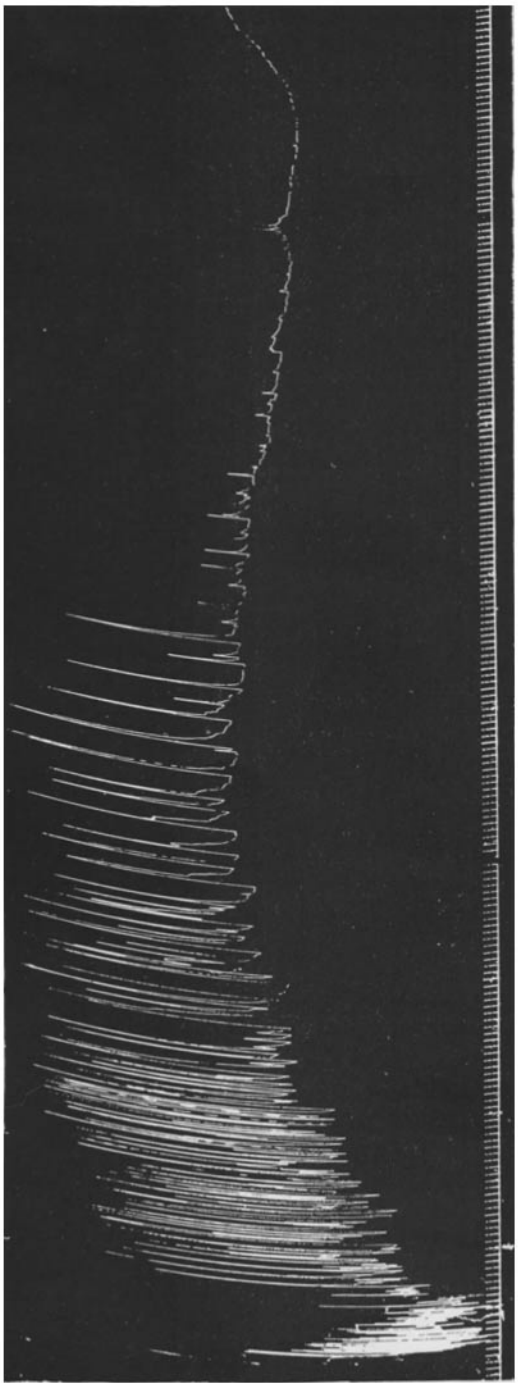

FIG. 2. *n*-Pentane.

n-Hexane.—The expectancy value is $\bar{\tau} = 417 \pm 34$ minutes. The mortality up to the expectancy value is $P(\bar{\tau}) = 0.63$.

n-Heptane.—The expectancy value is $\bar{\tau} = 788 \pm 59$ minutes. The mortality up to the expectancy value is $P(\bar{\tau}) = 0.57$ (Fig. 3).

It should be added that six experiments with *n*-octane gave an average lifetime of $\bar{\tau} = 2260$ minutes; four observations with *n*-decane (Fig. 4) gave $\bar{\tau} = 4450$ minutes; three experiments with *n*-dodecane and three with *n*-tetradecane showed practically the same results as the experiments with nujol, an expectancy value of 4800 minutes. Nine observations with *i*-octane (2,2,4-trimethylpentane) indicated an expectancy value of 1720 minutes; this last result is not essentially different from that obtained with *n*-octane. It merely supports the *n*-octane position.

Ten experiments with *n*-pentene (β,*n*-amylene) showed an average lifetime of only 5 minutes, more than 36 times shorter than with *n*-pentane, a value that may possibly be subject to small correction. More experiments will have to be carried out. Besides, the procedure of arranging the recording method takes too long compared with the actual average. But in no case will one arrive at an average of more than about 10 minutes.

The substances used form a definite arithmetic progression with their rising molecular weight. They are practically non-associated liquids and therefore the least complicated solvents for binary or ternary systems.

Computing the corresponding average lifetime against the rising molecular weight, we get a lifetime (mortality) curve of a particular kind (Fig. 5 *A*). This curve expresses a statistical function, the expectancy value, dependent on substances with continuously rising molecular weight, the $\bar{\tau}(M_n)$-curve. $\bar{\tau}$ is the expectancy value, M_n the respective molecular weight with n as the number of C atoms.

By graphic differentiation Fig. 5 *A* will furnish the corresponding distribution curve $\bar{\tau}'(M_n)$. However, we propose for Fig. 5 *A* the explicit form:

$$\bar{\tau} = \bar{\tau}_0(1 - e^{-\alpha M_n}) \tag{1}$$

Here $\bar{\tau}$ stands for the respective average lifetime, $\bar{\tau}_0$ the maximal value this expectancy value can attain; α should be a constant representing the decrease in mortality (or the increase in lifetime) as soon as the molecular weight rises by 14. The limiting conditions of equation (1) are: for

$$n = 0 \tag{2}$$

we get

$$\bar{\tau} = 0 \tag{3}$$

and for

$$n = \infty \tag{4}$$

i.e., for great molecular weight,

$$\bar{\tau} = \bar{\tau}_0 \tag{5}$$

FIG. 3. *n*-Heptane.

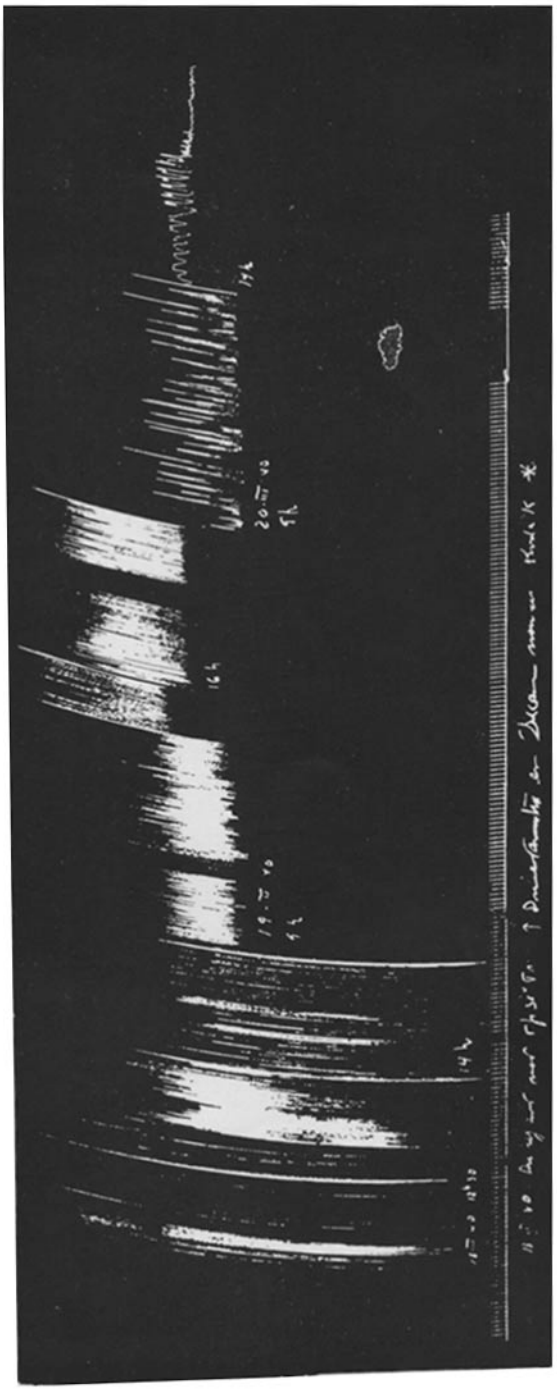

FIG. 4. n-Decane.

The differentiation of equation (1) furnishes the distribution function:

$$\bar{\tau}' = \alpha\bar{\tau}_0 e^{-\alpha M_n} = \alpha(\bar{\tau}_0 - \bar{\tau}) \tag{6}$$

Unfortunately, the values of the lifetimes for the range

$$n \lessapprox 10 \tag{7}$$

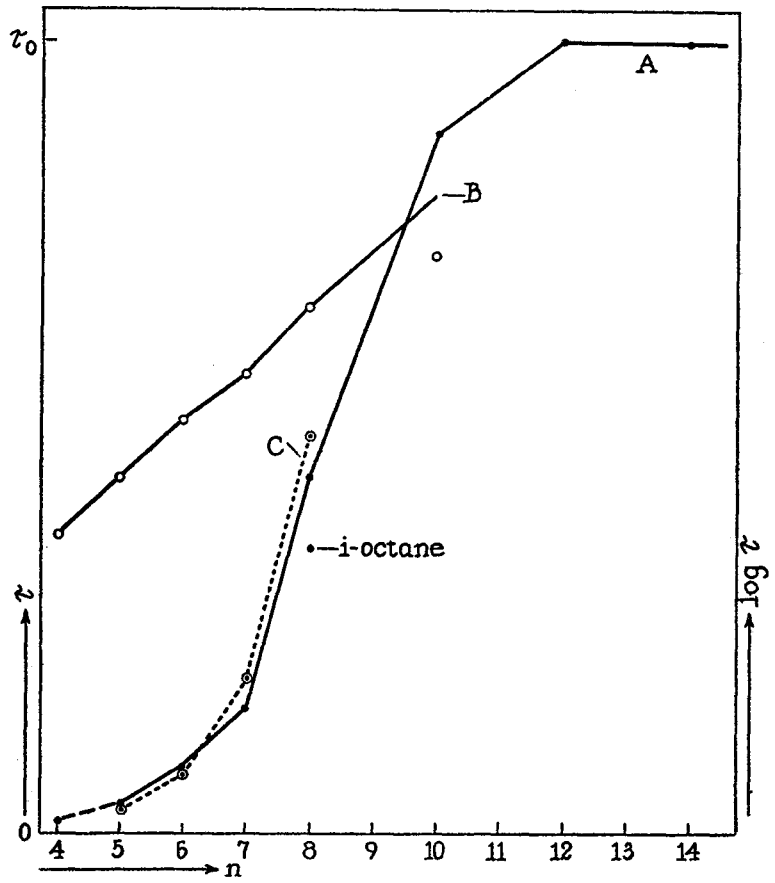

FIG. 5.

are not very precise. It is therefore impossible at present to discuss in detail the *whole* $\bar{\tau}(M_n)$ curve. On the other hand, the $\bar{\tau}$ values for the range

$$5 \lessapprox n \lessapprox 8 \tag{8}$$

are quite exact, so that we may pay closer attention to this part of the curve. Fig. 5 *A* seems to indicate that the values of the range in equation (8) follow the exponential function:

$$\bar{\tau} = e^{\alpha M_n} - 1 \tag{9}$$

a function closely related to equation (1).
For

$$n = 0 \tag{10}$$

we have as in equation (2)

$$\bar{\tau} = 0$$

The initial value of the function is zero, and it reaches this point with a disappearing tangent. We may state

$$\bar{\tau} + 1 = y \tag{11}$$

and get

$$\log y = \log (\bar{\tau} + 1) = \alpha\epsilon_0 M_n \tag{12}$$

with

$$\epsilon_0 = 0.43429.$$

TABLE I

Substance	τ (observed)	τ (calculated)	α
	min.	*min.*	
n-Pentane	183	144	6.9×10^{-2}
n-Hexane	417	381	$\pm$
n-Heptane	788	954	1.2×10^{-2}
n-Octane	2260	2294	

This $\log [\bar{\tau} + 1](M_n)$ function (Fig. 5 *B*) is a straight line. It hits reasonably close to the calculated points. From *n*-decane on, however, differences may be noted. As a mean value for α we find

$$\alpha = 6.9 \times 10^{-2} \pm 1.2 \times 10^{-2} \tag{13}$$

α was placed in equation (9) and $\bar{\tau}$ calculated. Fig. 5 *C* represents the curve. Table I shows the corresponding values.

Differentiating equation (9), we get, considering equation (11),

$$\frac{dy}{dM_n} - \alpha y = 0 \tag{14}$$

or

$$\frac{d\bar{\tau}}{dM_n} - \alpha\bar{\tau} - \alpha = 0 \tag{15}$$

the differential equation expressing the law at the basis of our observations. With rising M_n y, or $\bar{\tau}$ rises in proportion to the existing y, or $\bar{\tau}$. As soon as the molecular weight of the hydrocarbon liquid rises by the amount $\Delta M = M_{n+1} - M_n$ (by 14 or by one CH_2 group), the expectancy value rises proportionately

to the existing lifetime. The proportionality factor is the constant α. In consequence, with the molecular weight rising by equal values, the $\bar{\tau}$ must rise acceleratedly, for the exponential function goes to infinity very rapidly with constantly rising arguments. Thus the relation $\frac{\bar{\tau}_{n+1}}{\bar{\tau}_n}$ for $\Delta M = M_{n+1} - M_n$ remains constant.

$$\frac{\bar{\tau}_{n+1} + 1}{\bar{\tau}_n + 1} = \frac{y_{n+1}}{y_n} = e^{\alpha \Delta M_n} \tag{16}$$

As soon as the molecular weight rises in an arithmetic progression with the ratio ΔM, the lifetime $\bar{\tau}$ will rise in a geometric progression. We have

$$\frac{\log y_{n+1} - \log y_n}{\Delta M} \cdot \epsilon = \alpha$$

with (17)

$$\epsilon = 2.30259$$

As soon as the chain length rises by a definite amount, the average lifetime will rise in relation to the present one, regardless of the number of groups already present; *i.e.*, regardless of the molecular weight.

THEORETICAL PART

We assume that the higher the molecular weight, the longer the saturated, unramified chain, the fewer the molecules that will enter the animal.[2]

These quasi-linear hydrocarbon molecules consist of a definite number of practically equal parts, of CH_2- and CH_3 groups. The action of a molecule can thus—in the first approximation—be regarded as the sum of the actions of all these equal groups. The action of the molecules C_nH_{2n+2} is approximately the action of a unit of $n CH_2$ groups.[3] The number of CH_2 groups on the spot is, then, responsible for the action of this kind of molecule. Consequently, if the molecular weight increases by 14, not only fewer molecules will enter, but also fewer CH_2 groups. The problem of our $\bar{\tau}(M_n)$ curve thus becomes the $\bar{\tau}(CH_2)$ problem.

Obviously, it is not possible to determine how many molecules or groups enter the *Ascaris* under given experimental conditions. But it is possible to make a limited deduction. As soon as we go from the liquid with molecular weight M_n over to that with molecular weight M_{n+1}, where n and $n + 1$ represent the number of C atoms per

[2] In other words, the higher the number of CH_2 groups within the hydrocarbon molecule, the stronger the effort of the molecule to leave the water phase.

[3] We omit here the influence of the geometric form of these molecules. This factor as well as others, though theoretically well known, can nevertheless not be taken into practical consideration.

molecule, then the number of molecules that have entered into the body must be

$$z_{n+1} < \frac{\varkappa}{n+1} \cdot z_n \tag{18}$$

where z_{n+1} and z_n each express the number of molecules of the kind $C_{n+1}H_{2(n+1)+2}$ and C_nH_{2n+2}, respectively. For under the condition

$$z_{n+1} = \frac{\varkappa}{n+1} \cdot z_n \tag{19}$$

an equal number of CH_2 groups from both kinds of molecules would enter. Suppose 100 molecules of *n*-pentane have penetrated the organism. From *n*-hexane, then, less than 83 molecules will enter, from *n*-heptane less than 71, from *n*-octane less than 60, and so on.

So, with a rising molecular weight the expectancy value will increase. Close to *n*-undecane or *n*-dodecane the relationship appears to cease. Before an explanation of this fact can be attempted, more information concerning the physical behavior of these molecules is needed. A closer biologic investigation of this area will also be necessary.

At equilibrium and under ordinary conditions of state the vapor phases of our hydrocarbon liquids of the range (equation (8)) may obey the ideal gas laws.[4] The vapor pressure π is then equal for both phases; the free energy ψ of the corresponding liquid is known up to an isothermic constant π_0. As with Ferguson (12) ψ may serve as thermodynamic potential function.[5] We have

$$d\psi = RTd \ln \pi \tag{20}$$

It is known (Young (17)) that the vapor pressure within the methane series diminishes with rising molecular weight. Some other physical constants do the same or just the opposite.

The difference in free energy for the liquids $C_{n+1}H_{2n+4}$ and C_nH_{2n+2} at a given temperature is then according to equation (20):

$$\Delta\psi = \psi_{n+1} - \psi_n = RT \int_n^{n+1} d \ln \pi \tag{21}$$

$$\Delta\psi = RT \epsilon \log \kappa \tag{22}$$

with

$$\kappa = \frac{\pi_{n+1}}{\pi_n} = \frac{V_n}{V_{n+1}} = \frac{M_n \cdot \sigma_n}{M_{n+1} \cdot \sigma_{n+1}} \tag{23}$$

$$\kappa = 8.92 \times 10^{-1} \pm 6.4 \times 10^{-3} \tag{24}$$

where π_{n+1} and π_n are the respective vapor pressures, V_{n+1} and V_n the respective molar volumes, M_{n+1} and M_n the respective molecular weights, and finally σ_{n+1} and σ_n the specific volumes. All the necessary numerical values are presented in Table II.

[4] If that should not seem acceptable, then the van der Waals equation may be used.

[5] Any other of the thermodynamic potentials would have served too. It would have been the same with the "molar entropy." But there was no apparent advantage in it. Later on we tried to use even the vapor pressure π itself.

Consequently we have

$$\Delta\psi = RT\,\epsilon \log \frac{M_n}{M_{n+1}} + \gamma \tag{25}$$

$$\Delta\psi = 7.08 \times 10^{1} \pm 4.4 \times 10^{0} \text{ cal. mol}^{-1} \tag{26}$$

From (20) we deduce:

$$\psi_n = -RT\,\epsilon \log \frac{V_n}{RT} + \pi_0 \tag{27}$$

or

$$\psi_n = -RT\,\epsilon \log M_n \cdot \sigma_n + \pi'_0 \tag{28}$$

where

$$\pi'_0 = RT\,\epsilon \log RT\,\pi_0 \tag{29}$$

TABLE II

Substance	$\frac{M_n}{M_n+1}$	σ_n	$\frac{\sigma_n}{\sigma_n+1}$	$\kappa = \frac{\pi_n+1}{\pi_n}$	$\Delta\psi$
n-Pentane		1.644			
	0.8372		1.0572	0.8851	Average value:
n-Hexane		1.5551			
	0.8600		1.0300	0.8858	70.84 ± 4.4
n-Heptane		1.4961			cal. mol.$^{-1}$
	0.8772		1.0312	0.9045	
n-Octane		1.4508			

Average for:

(1) $\frac{\sigma_n}{\sigma_{n+1}} = 1.0373 \pm 9.0 \times 10^{-3}$

(2) $\kappa = 8.92 \times 10^{-1} \pm 6.4 \times 10^{-3}$

The dependence of the free energy on the molecular weight or on the molecular volume may be found described in equations (27) or (28). The effort of the molecules with $(n+1)$ CH_2 groups to leave their phase is smaller by 70.84 cal. mol^{-1} than that of the molecules with only n CH_2 groups. All this holds, of course, for both liquids under identical conditions and for the range (equation (8)). Dividing equation (27) by $N = 6.06 \times 10^{23}$, the Avogadro number, we get

$$\Delta\psi^{(CH_2)} = \frac{\Delta\psi}{N} = kT\,\epsilon \log \frac{M_n}{M_{n+1}} + \gamma' \tag{30}$$

$$\Delta\psi^{(CH_2)} = 1.17 \times 10^{-22} \text{ cal.} = 3.05 \times 10^{-3}\, eV, \tag{31}$$

where $k = 1.372 \times 10^{-16}$ erg. grad^{-1} is the Boltzmann constant. Equation (31) gives the rise in free energy for one molecule, provided this molecule has n groups CH_2 instead of $(n+1)$ groups. Since κ is rather constant throughout (equation (8)) (Table II), it will be the same with $\Delta\psi$.

This consideration would appear to explain our biologic observations and the respective conclusions we have drawn from them.

The energy that one of the hydrocarbon molecules exercises on its immediately surrounding area was regarded, in the first approximation, as the sum of the energies of all its single CH_2 groups. The potential between these molecules rises simply as the number of their CH_2 groups rises. It is defined for each group by the Wang-London (13) function ($\phi = -Cr^{-6}$) of the van der Waals forces alone.[6] To take a molecule out of its liquid requires the breaking of only this kind of bond, and these are not very strong (about 500 cal. mol^{-1}) at room temperature. So the evaporation heat of substances such as we used will be relatively low[7] (as must also be their boiling point because of Pictet-Trouton's rule) and proportionate to the number of CH_2 groups of the molecule. Table III shows that $\overline{\Delta\lambda}$ is practically constant throughout our range (Equation 8). To bring a molecule with $(n + 1)$ CH_2 groups from the liquid into the vacuum requires roughly 0.6 Kcal. mol^{-1} [8] more than for one with n groups.

TABLE III

Substance	Molecular weight	Boiling point	Heat of evaporation λ
		°C.	*Kcal. mol*$^{-1}$
n-Pentane	72	36	6.5
n-Hexane	86	68	7.2
n-Heptane	100	98.2	7.8
n-Octane	114	125.6	8.4
n-Decane	128	174.0	9.4

The numbers are calculated from Landolt-Börnstein's Tables:

$$\overline{\Delta\lambda} = \overline{\lambda_{n+1} - \lambda_n} = 6.3 \times 10^{-1} \pm 3.4 \times 10^{-2}$$

This explains the rather regularly decreasing vapor pressures and increasing boiling points, etc., with rising molecular weight of the hydrocarbons in the methane series.

With all that, are given certain indications of the physical behavior of the liquids used in our experiments. Qualitatively, this seems to coincide with their biological actions. The higher the molecular weight, the smaller the free energy according to equation (28), the longer the average lifetime according to equation (9), and the smaller the concentration of CH_2 groups within the organism according to equation (18), and *vice versa*. The following considerations, however, make it apparent that the matter is not so simple, though these physical conditions are still of primary importance.

[6] We refer to our footnote 3 regarding the influence of the geometric and other quantitatively still unknown factors upon the interaction of these thread-like molecules.

[7] *n*-Pentane, with its molecular weight of 72.0, has a boiling point at 38°C. and the evaporation heat of 6.5 Kcal.mol^{-1} at boiling point. Water, a molecule with a strong dipole (1.79D.) and other electrostatic effects in its vicinity, has the molecular weight 18.0, the boiling point at 100°C., and an evaporation energy of 9.73 Kcal.mol^{-1}.

[8] That is the "total" heat of evaporation. In our numbers is thus included the work for the occurring change in the specific volume.

The expectancy value $\bar{\tau}$ according to equation (9) depends on the molecular weight in an exponential way. The free energy ψ, however, as expressed in equations (20) and (28), is a logarithmic function. While the lifetime rises rapidly with rising chain length, the free energy decreases slowly. When, with molecular weight approaching zero, the lifetime approaches zero too asymptotically, then the free energy approaches negative infinity very rapidly.

This may suggest that we take as characteristic function the vapor pressure π instead of the free energy ψ as a measure for the effort of the molecules to abandon their phase. We will then get two exponential functions that can be directly compared. For the lifetime we arrived at in (equation (9)):

$$\bar{\tau}_n = e^{\alpha M_n} - 1 \tag{9'}$$

But the vapor pressure from (20) is:

$$\underline{\pi} = e^{\frac{\psi}{RT}} \tag{32}$$

where $\underline{\pi}$ represents the vapor pressure in relation to a convenient standard. Equation (28) gives for the free energy

$$\psi_n = -RT\,\epsilon \log M_n \cdot \sigma_n + \pi_0' \tag{28'}$$

So equation (32) becomes

$$\underline{\pi_n} = e^{-\epsilon \log M_n\, \sigma + \pi''_0} \tag{33}$$

where there is

$$\pi''_0 = \epsilon \log RT\, \pi_0 \tag{34}$$

The lifetime $\bar{\tau}_n$ and vapor pressure $\underline{\pi_n}$ depend on the molecular weight in two essentially different ways—not merely opposite ones. Here the action of the organism may become evident with the interference of velocity procedures. Mere equilibrium considerations will scarcely be sufficient to explain the marginal value $\bar{\tau}_0$ of the $\bar{\tau}(M_n)$ function in equation (1), as where there is:

$$\bar{\tau} = \bar{\tau}_0 \tag{35}$$

With a homogeneous system the links of the methane series will necessarily behave differently than in a heterogeneous system. In the first case the interactions will, roughly speaking, depend directly on the number of CH_2 groups within the respective molecules (Table III). In the second one, however, it is the vapor pressure, etc., of the respective molecule that decides its action on the neighboring phases, and this action must be precisely inverse to the number of CH_2 groups within the molecule. So the Wang-London energy that, with rising number of CH_2 groups, holds these molecules more closely together, raises their cohesion, their boiling point, their evaporation heat, their viscosity, etc., at the same time lowers their capability to leave their phase. The first situation will arise as soon as homologous series such as ours act in biologic liquids like serum or in protoplasm, at least within their respective intermi-

cellar fluids. The second will occur where such molecules are in contact with cells.

Within an organism both actions will occur simultaneously. But it may happen, as it did in our experiments, that one action dominates. With a heterogeneous system we shall observe the particular effect growing weaker with rising n. In the homogeneous system, however, under the same conditions, the effect will grow stronger. Conversely, shall we thus finally be able to decide, whether a given kind of substances acted essentially within homogeneous surroundings or within heterogeneous ones?

The small difference in efficiency of the ramified molecule as compared with the straight one of the same number of C atoms is, for the most part, not surprising. We have become accustomed to it from colloid chemistry and other experiences. It may be, however, that further experiments will be able to detect something here despite colloid chemical experiences.

The described action of the double bond within a straight chain also was to be expected. The double bond usually acts very much like a polar bond only less strongly. Characteristic polar molecules such as oleic acid—which contain a double bond within their long chain and form monomolecular layers on water—demonstrate this. It is also known from experiments on solubility. The relatively high polarizability of the double bond may account for all this.

The papers of Brink and Posternak (1) and of Chadwick and Dethier (2) are more or less closely connected with our own.

CONCLUSIONS

1. The biologic experiments with the links of the methane series—*n*-pentane, *n*-hexane, *n*-heptane, *n*-octane, *i*-octane, and pentene—gave these qualitative results: (*a*) The higher the number of CH_2 groups, the longer the chain, the longer the average lifetime of the animal. (*b*) The ramified chain does not appear to act differently from the saturated straight chain with the same number of C atoms. (*c*) One double bond within the chain shortens the lifetime to a considerable degree.

2. The quantitative discussion shows that the lifetimes depend exponentially on the molecular weight.

3. Qualitatively the hypothesis is supported that with rising molecular weight the concentration of CH_2 groups within the animal diminishes according to the vapor pressure or the thermodynamic potential. However, lifetime and these physical properties obey different functions.

4. These physical properties are of high biologic importance. But they are not sufficient to explain the biologic effects quantitatively.

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