the whole molecular periphery. (The total electronic distribution in the free radical may be obtained by adding the distribution of the odd electron to that of FMN.)

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[15] See, e. g., n. 1, chap. 10.

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*ON THE SIZE OF GENETIC DETERMINANTS IN PNEUMOCOCCUS AND THE NATURE OF THE VARIABLES INVOLVED IN TRANSFORMATION**

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INTRODUCTION

The process of bacterial transformation is a complex one involving many steps, some of which are still obscure. Whenever any property of the transforming DNA is altered, each step can be expected to respond in a characteristic way. Thus, in the absence of a quantitative formulation of the variables involved, it has been

impossible to interpret unambiguously physical studies of transforming DNA with respect to their biological implications. The recent derivation[1] of such equations, however, permits the design of experiments which give more specific information. We present here simultaneous absorption and transformation measurements using quadruply marked pneumococcal DNA degraded by mechanical shear,[2, 3] a method which produces only double-chain scissions. From these data we have been able to obtain information concerning the molecular-weight dependence of variables involved in DNA adsorption, absorption and incorporation into the genome, the size of genetic determinants (markers), and the pneumococcal surface. The term "marker" is used herein to designate the (minimum) segment of DNA that must be incorporated into the genome in order to effect transformation to the new genotype. Markers do not, therefore, necessarily constitute the entire genetically functional unit or gene, an absolute estimate of whose size might be obtained from experiments with linked markers.

METHODS AND MATERIALS

DNA.—DNA was prepared from a rough strain of *Diplococcus pneumoniae*, designated R6,[4] which had been transformed to sulfanilamide (50 μg/ml), streptomycin, micrococcin, and erythromycin (0.25 μg/ml) resistance. After lysis with deoxycholate in 0.1 *M* citrate–0.15 *M* NaCl, preparations were deproteinized in one of three ways: with chloroform-octanol (Sevag), with duponol,[5] or with saturated NaBr.[6] Mutually consistent results were obtained using DNA prepared in all three ways. Most of the measurements reported here were performed with a single Sevag preparation.

DNA labeled with P^{32} was prepared by growing the same quadruply marked strain in a tryptose-glucose medium to which radioactive phosphate had been added after removal of nonlabeled phosphate.[7] The specific activity of the final product was 12 μc/mg.

Degradation.—A spray procedure[2] was used to degrade the DNA by means of mechanical shear. This method produces decreases in molecular weight by means of double-chain scissions; no structural changes (denaturation) can be detected.[3] Variation in the shear rate produced samples of different average molecular weights. Experimental details are presented elsewhere.[3]

Physical Measurements.—Sedimentation of each sample was carried out at a concentration of 0.03 mg/ml in 0.2 *M* salt, in a Spinco Model E Ultracentrifuge equipped with ultraviolet optics, and sedimentation distributions were calculated. An empirical relationship between the sedimentation constant at the peak and the molecular weight obtained from light scattering has been developed,[3] enabling the conversion of sedimentation to molecular-weight distributions. These distributions become narrower as the molecular weight decreases. Number-average molecular weights were calculated from the molecular-weight distributions. Weight-average weights, similarly calculated, agreed with those obtained from light-scattering measurements. The latter were carried out in a Brice-Phoenix light-scattering photometer using a conical cell. A discussion of the interpretation of light-scattering results, together with experimental details and a more extensive presentation of the physical properties of sheared DNA will be found elsewhere.[3]

Transforming Activity Assays.—Frozen sensitized cultures[8] of *D. pneumoniae*

R6 were employed as recipients for transformation, following the general procedure of Hotchkiss.[9] After exposure to DNA at 30° C. in supplemented casein-hydrolysate medium, the cells were incubated with DNase at 37° until maximum expression of the new character had occurred. Extreme care was exercised in making dilutions for scoring: high-precision pipettes were used, the tips were freed from adhering solution before delivery, and, in all but one step, all samples were diluted identically. Concentrations were adjusted so that ordinarily from 10 to 50 colonies were counted in each tube. DNA concentration-activity curves were obtained over a thousand-fold range for samples of several different molecular weights. Ordinarily, assays were performed at the plateau (excess DNA), using three concentrations as a check; each concentration was scored in triplicate. Each assay was repeated independently from one to five times, or until the majority of the results agreed within a narrow range; widely differing values occurred infrequently and were discarded. The average values used were thus accurate to ±10 per cent or, in most cases, considerably better. The number of cells transformed ranged from about 15 per cent for streptomycin resistance to about 0.2 per cent for erythromycin resistance.

Absorption Measurements.—A procedure identical with that used for transformation assays, up to the point of DNase addition, was followed. Then 0.5 mg. of DNase (solution containing $MgCl_2$) was added to each tube, followed by incubation at 37° C. for 25 minutes. Thereafter the temperature was maintained near 0° C., and the bacteria were washed with cold medium until no more radioactivity could be removed (esssentially the same procedure as that used by Lerman and Tolmach[7]). The pellet of bacteria was then counted in an end-window Geiger-Müller counter. The counting precision was 3 per cent.

RESULTS

I. The number of transformed bacteria obtained in a quantitative assay of DNA transforming ability is given by[1]

Number of transformants $= Nv_{tr}t$

$$= \frac{Nt\Sigma([B_0]\alpha k_m K_m[D]X)_i}{1 + \Sigma(K_m[D]X)_i + \Sigma(K_{m+c}[D])_i} \approx \frac{t\overline{[B_0]\alpha k_m K_m}[D]M_n}{1 + \bar{K}_{m+c}[D]}\left(\frac{NX}{M_n}\right), \qquad (1)$$

where v_{tr} is the (molar) rate of initiation of transformation,[10] and is constant under the conditions used; N is Avogadro's number; t is time; the subscript i denotes a particular molecular-weight species; $[B_0]$ is the total concentration of (effective) bacterial sites; α is the probability that a marker in an absorbed DNA molecule will be incorporated into (or interact with) the genome; k_m is the absorption rate constant for DNA molecules containing the marker of interest; K_m is the inverse Michaelis constant for marked DNA molecules; K_{m+c} is the inverse Michaelis constant for all the (marked + competitor) DNA molecules (both K's may be regarded as binding constants[1]); bars denote number averages; $[D]$ is the (molar) concentration of *unbound* DNA; M_n is the number-average molecular weight of the DNA; X is the mole fraction of marked molecules; NX/M_n is the number of markers per gram of DNA. In most cases it is this last quantity which is of interest. The quantities $\overline{[B_0]\alpha k_m K_m}$ and $\bar{K}_{m+c}$ are number averages, since DNA is polydisperse and each variable may be a function of molecular weight.

When bacterial sites are in excess (on the linear part of the transformants versus DNA curve), equation (1) reduces to

$$(\text{No. trans.})_{\text{linear}} \propto M_n \overline{[B_0]\alpha k_m K_m}\ [D] \left(\frac{NX}{M_n}\right). \tag{2}$$

When DNA is in excess (on plateau of the curve),

$$(\text{No. trans.})_{\text{plateau}} \propto \frac{M_n \overline{[B_0]\alpha k_m K_m}}{\bar{K}_{m+c}} \left(\frac{NX}{M_n}\right). \tag{3}$$

If one DNA molecule, regardless of molecular weight, is bound by one bacterial site (hereafter called "molecular binding"), $[B_0]$ is constant and may be omitted from equations (2) and (3). If, on the other hand, a single DNA molecule requires a variable number of bacterial binding sites (e.g., equal to the number of nucleotide pairs or some other unit group comprising the molecule) (hereafter called "unit binding"), $[B_0]$, which is defined as the concentration of *molecular* binding sites, is a function of the molecular weight of the DNA. Then

$$[B_0]_i = \frac{[S_0]}{n_i} \propto \frac{[S_0]}{M_i} \propto \frac{1}{M_i},$$

where $[B_0]_i$ is the total concentration of *molecular* binding sites for DNA molecules of molecular weight M_i; $[S_0]$ is the total concentration of *unit* binding sites and is therefore constant; n_i is the number of binding units in the ith molecules; M_i is the molecular weight of the ith molecules. In this case, the quantity $\overline{[B_0]\alpha k_m K_m}$ in equations (2) and (3) may be replaced by $\alpha k_m K_m / M$.

We have derived an equation (see Appendix) which expresses the molecular-weight dependence of the number of markers in unit weight of DNA (NX/M_n). As the molecular weight decreases, markers are destroyed by cleavage, and the fraction of the markers remaining intact is

$$\left(\frac{NX}{M_n}\right) \Big/ \left(\frac{NX}{M_n}\right)_0 = \left[\frac{\bar{M}_0(W - G)}{\bar{M}_0 - W + G}\right] \frac{1}{M_n} + \left[\frac{\bar{M}_0}{M_0 - W + G}\right]. \tag{4}$$

Here $(NX/M_n)_0$ is the number of markers per unit weight of original, undegraded material; W is the "molecular" weight of a marker; $\bar{M}_0$ is the original number-average molecular weight of the DNA; G is the weight of a nucleotide pair. Since these are all constants, we may write[11]

$$\frac{NX}{M_n} = \frac{a}{M_n} + b, \tag{5}$$

where a and b are constants which can be defined in terms of the marker weight, or size. Expression (5) can be substituted in equation (2) or (3), giving, for the plateau situation,

$$\text{No. trans.} \propto bZM_n + aZ, \tag{6}$$

where

$$Z = \frac{\overline{[B_0]\alpha k_m K_m}}{\bar{K}_{m+c}}.$$

If Z is independent of molecular weight, a plot of the number of transformants (or the per cent transforming activity) versus M_n will be linear, and the marker size can be calculated from either the slope or the intercept.

DNA obtained from quadruply marked pneumococcal cells was progressively degraded by mechanical shear to yield a series of samples differing in molecular weight. The relative transforming activity (relative number of transformants obtained under standard conditions) of this material with respect to sulfanilamide, streptomycin, micrococcin, and erythromycin resistance is plotted against M_n in Figures 1 and 2. Each point is the average of from two to six separate deter-

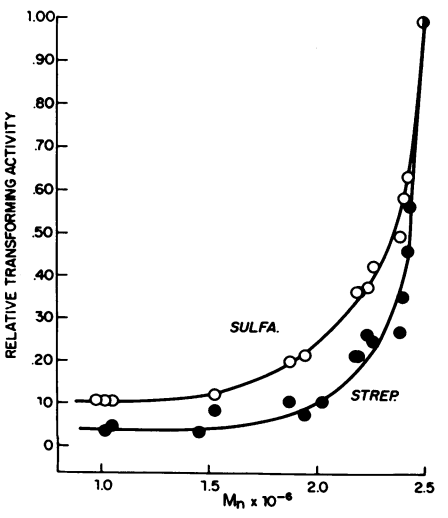

FIG. 1.—Sulfanilamide-and streptomycin-resistance transforming activity as a function of the number-average molecular weight of the DNA.

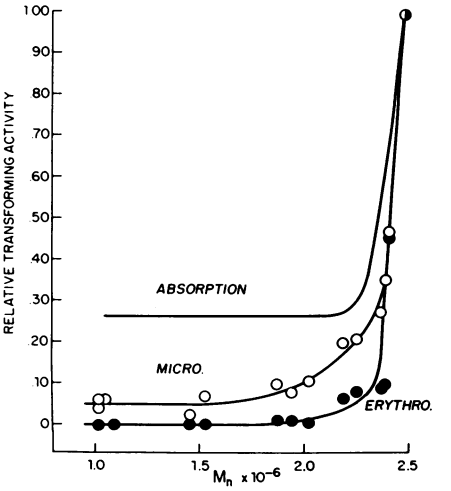

FIG. 2.—Micrococcin- and erythomycin-resistance transforming activity as a function of the number-average molecular weight of the DNA. The curve for relative weight of DNA absorbed versus M_n is superimposed on the same scale for comparison.

minations and is accurate to ± 10 per cent or better. All determinations were carried out in the plateau region of excess DNA. The curves for the four markers clearly differ, and these differences are significant because all data were obtained with the same DNA preparation. However, all the markers show a sharp initial drop, followed by a leveling to constant activity at lower molecular weights. To gain insight into the molecular-weight dependence of the activity, the latter was plotted against $1/M_n$. In this case, too, nonlinear curves of the same general shape were obtained. For the case of unit binding, it has been shown above that $[B_0] \propto 1/M$. Equation (6) then becomes approximately: No. trans. $\propto bZ + aZ/M_n$, where Z no longer contains $[B_0]$. Because of the observed curvature of activity versus both M_n and $1/M_n$, Z, in equation (6), apparently depends on molecular weight, and this dependence cannot be attributed to $[B_0]$ alone.

II. The weight of DNA irreversibly[7] absorbed by bacteria is expressed by[1]

$$\text{Weight absorbed} = t\Sigma M_i v_{\text{abs}i} = \bar{M}_{\text{abs}}\, v_{\text{abs}} t = \frac{t\Sigma(M[B_0]k_{m+c}K_{m+c}[D])_i}{1 + \Sigma(K_{m+c}[D])_i} = \frac{t\,\overline{M[B_0]k_{m+c}K_{m+c}}\,[D]}{1 + \bar{K}_{m+c}[D]}, \quad (7)$$

where v_{abs} is the (molar) rate of absorption; $\bar{M}_{abs}$ is the number-average molecular weight of the *absorbed* DNA; k_{m+c} is the absorption rate constant; and all other symbols were defined previously. In the region of linear concentration response, this equation reduces to

$$(\text{Wt. abs.})_{\text{linear}} \propto \overline{M[B_0]k_{m+c}K_{m+c}}\,[D]; \tag{8}$$

in the plateau region,

$$(\text{Wt. abs.})_{\text{plateau}} \propto \frac{\overline{M[B_0]\,k_{m+c}\,K_{m+c}}}{\overline{K}_{m+c}}. \tag{9}$$

In these equations, $\overline{M\,[B_0]k_{m+c}\,K_{m+c}}$ becomes $\overline{Mk_{m+c}K_{m+c}}$ or $\overline{k_{m+c}K_{m+c}}$, depending on whether the bacterial sites bind DNA on a molecular or unit basis, as discussed above. In the latter case, it can be seen that if k_{m+c} and K_{m+c} are constant, the weight absorbed will be independent of the molecular weight of the DNA (curve *A*, Fig. 3); in the former case, under the same conditions, a sloping, linear plot of weight absorbed versus M_n will be obtained (curve *B*, Fig. 3).

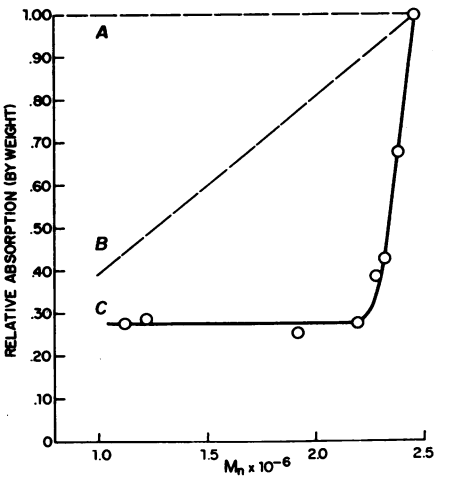

FIG. 3.—Curves *A* and *B:* theoretical curves discussed in the text. Curve *C:* relative weight of DNA absorbed by pneumococci, as a function of the number-average molecular weight of the DNA.

We have measured the absorption by pneumococci of P^{32}-labeled pneumococcal DNA, containing the same four markers and degraded as described above, under the conditions used for assay of transforming activity. Activity determinations on this material gave essentially the same results as were obtained with unlabeled DNA. The absorption results, obtained in the plateau region, are presented in Figure 3, curve *C*. This nonlinear curve differs from both *A* and *B*, showing that k_{m+c} and/or K_{m+c} are dependent on molecular weight. The absorption curve bears a striking resemblance to the activity curves in its initial drop and subsequent leveling, but they are not superimposable, as can be seen in Figure 2.

III. Inhibition experiments[1] have previously indicated that the binding constant of DNA to pneumococci decreases with the molecular weight of the DNA. A more detailed investigation of the relationship is presented here. The transforming activities of three DNA samples (M_n = 2.5, 2.2, and 1.0 × 10^6) were assayed at a series of DNA concentrations, and the reciprocal numbers of transformants obtained were plotted against the reciprocal DNA concentrations (for cases where the relative amount of DNA bound was negligible). It can be seen from equation (1) that division of the intercept by the slope of each curve gives $\overline{K}_{m+c}$. The values obtained were approximately 8500, 2500, and 2500 l/mol for M_n = 2.5, 2.2, and 1.0 × 10^6, respectively. Thus the molar binding constant appears to have a molecular weight dependence similar to that of absorption (Fig. 3, *C*), with constancy in the same region.

DISCUSSION

As the molecular weight drops, an initial, sharp decrease in both the weight and the number of moles of DNA absorbed at the plateau by pneumococci has been observed (Fig. 3, *C*), followed by an abrupt, perhaps discontinuous, change in behavior at $M_n = 2.2 \times 10^6$. The same appears to be true of the binding constant ($\bar{K}_{m+c}$). These observations can be interpreted most simply in terms of two types of sites on the bacterial surface, one of which (*I*) is capable of absorbing only relatively high-molecular-weight DNA. Since the absorption measurements were made under conditions in which the bacterial sites are saturated, the absorption rate constant $(k_{m+c})_I$ (rather than the binding constant) must be responsible for the sharp drop and must therefore decrease to zero in the vicinity of $M_n = 2.2 \times 10^6$.

If there are, in fact, two types of DNA absorption sites on the surfaces of competent pneumococcal cells, equation (1) becomes the sum of two terms,

$$\text{No. trans.} = t\left[\frac{\alpha[B_0]_I k_{m_I} K_{m_I}}{1 + \bar{K}_{(m+c)I}[D]} + \frac{\alpha[B_0]_{II} k_{m_{II}} K_{m_{II}}}{1 + \bar{K}_{(m+c)_{II}}[D]}\right][D]M_n\left(\frac{NX}{M_n}\right) \quad (10)$$

the first of which equals zero when $M_n \leqslant 2.2 \times 10^6$. Thus the plots of 1/no. trans. versus $1/[D]$, mentioned above, yield $\bar{K}_{(m+c)_{II}}$ for the lower-molecular-weight DNA samples, while the value of "$\bar{K}_{m+c}$" obtained for $M_n = 2.5 \times 10^6$ is not really a binding constant at all. The decrease in this apparent binding constant between $M_n = 2.5 \times 10^6$ and $M_n = 2.2 \times 10^6$ is therefore indicative only of some change in absorption behavior.

A comparison of equations (3) and (9) (let us consider these for the moment as describing the site I component), for transformation and absorption at the plateau, shows that a sharp initial drop in absorption will be reflected as a drop in transformation, although the decrease need not be parallel; in addition to possible decreases in α and NX/M_n, which affect only the transformation measurement, we can conclude from the fact that k_{m+c} (and possibly K_{m+c}) decrease with molecular weight that k_m and possibly K_m also decrease (since marked molecules $[m]$ are included in the total ensemble $[m + c]$ and differ from it, with respect to absorption, only in that they may have a higher average molecular weight). Their rates of decrease need not be the same, however. Furthermore, even if $\overline{k_m K_m} = \overline{k_{m+c} K_{m+c}}$, as will be true if there is no marker destruction, the initial slopes of the transformation and absorption curves will differ if (*a*) M_n and $\bar{M}_{abs}$[12] decrease at different rates or (*b*) α is not constant. Thus *a large part, although not necessarily all, of the observed loss in transforming activity can be attributed to decreased absorption of DNA.*

The shapes of the absorption and transformation curves are, of course, dependent on the polydispersity of each sample of DNA. It should be noted that the steepness of descent and suddenness of inflection in these curves results in part from the type of degradation employed, i.e., nonrandom cutting of the larger molecules, which leads to progressive narrowing of the molecular-weight distribution as M_n decreases. Random degradation would undoubtedly have produced more gradual curvature.

We shall now consider the molecular-weight range where both absorption and transformation are constant for all markers. In this range, only type II sites can

absorb DNA, and equation (10) reduces to equation (1); therefore, all constants here are those for type II sites. Since the (plateau) absorption (eq. [9]) is constant,

$$\bar{K}_{m+c} \propto \overline{M[B_0]k_{(m+c)}\, K_{m+c}}, \tag{11}$$

and therefore $M_i[B_0]_i k_{(m+c)i}$ is constant. Thus $[B_0]_i\ k_{(m+c)i} \propto 1/M_i$.[13] Analogously, a similar relation holds true when only marked molecules are considered: $[B_0]_i\ km_i \propto 1/M_{mi}$. Substituting this in equation (3) for plateau transformation, substituting a constant for the number of transformants, and rearranging gives

$$\frac{NX}{M_n} \propto \frac{1}{M_n} \frac{\bar{K}_{m+c}}{\overline{\alpha\, K_m/M_m}},$$

where M_m is the molecular weight of marked DNA molecules only.

It has already been shown that the binding constant does not change in this region; therefore, $K_{mi} = K_{(m+c)i} = \bar{K}_{m+c}$, and

$$\frac{NX}{M_n} \propto \frac{1}{M_n} \frac{1}{\overline{\alpha/M_m}}. \tag{12}$$

The number of markers per unit weight of DNA, NX/M_n, may decrease or remain constant, but it cannot increase during degradation. It can be shown, however, that the right side of equation (12) can only increase or remain essentially constant.[14] Therefore, both sides of equation (12) must be essentially constant in the range of constant absorption and transformation. But, since equation (5) predicts that NX/M_n is a linear function of $1/M_n$, constancy in any molecular-weight range necessitates constancy throughout. Thus the number of sulfanilamide, streptomycin, micrococcin, and erythromycin markers per gram of DNA is essentially unchanged by degradation, and *the weight of a marker* (W, in eq. [4]) *must therefore be small—possibly of the order of several nucleotide pairs.*

It can be shown that α must also be constant in this low-molecular-weight range.[14] There is no reason to suppose, however, that the dependence of α on molecular weight must be linear or must be the same for all markers. On the contrary, the lack of congruity between the transforming activity curves for four different markers (Figs. 1 and 2) having the same size (within experimental error) and absorbability can be attributed only to a differing functional dependency of α on molecular weight for each marker. Thus α must, in general, decrease at first and then level off during degradation; but, since each marker has to interact with a different part of the genome, it is not surprising that α for each behaves somewhat differently with respect to changes in molecular weight. Since α may also vary with the intramolecular position of the marker, it must be considered an average even when it corresponds to a fixed molecular weight.

CONCLUSIONS

The four pneumococcal markers investigated here have been found to be very small. It is appropriate, at this point, to inquire into the physical significance of the genetic determinant or marker, defined here as the minimum segment of DNA that must be incorporated into the genome in order to effect transformation to the new genotype. Thus the marker must consist of a sequence of nucleotides, the

first and last of which are the most widely separated nucleotides that differ from the corresponding allelic sequence already possessed by the recipient cell; that is, it comprises the mutation or set of mutations that gave rise to the genotype in question. As such, its size ought to be of the same order of magnitude as that of the muton. Benzer[15] defines a muton as the smallest element that, when altered, can give rise to a mutant form of the organism. His estimate of muton size, based on biological recombination studies, is about five nucleotides, which is in happy agreement with the physicochemical conclusions presented here.

The decrease in α with molecular weight suggests the thesis that the greater the degree of similarity of the entering to the resident allelic DNA, the greater the probability of its interacting with the appropriate locus.

Rather than consider discontinuous behavior for rate (k) or binding (K) constants as the molecular weight of DNA decreases, we have postulated the existence of two types of absorption sites on the surface of the pneumococcus. The possibility has been discussed that a single site may adsorb either an entire DNA molecule or only a unit thereof of fixed size, so that in the latter case a (variable) number of sites is occupied by each molecule. The relative values determined for k and K at low molecular weights suggest that a type II site adsorbs an entire molecule but does so by interacting with only a limited portion of it at a time.[16] Type I sites are probably also "molecular" but interact with the entire molecule.[16] A difference in absorption mechanism can therefore be expected for the two types of site.

Comparison with Previous Work.—The considerations presented here are helpful in the interpretation of earlier work based on the application of target theory to radiation[17] and sonication[18] data. These studies produced estimates of the "minimum functional unit," "critical size," etc., which have been variously interpreted as the size of the markers, the minimum size for adsorption, absorption, resistance to intracellular DNA, or incorporation into the genome, or combinations thereof. Aside from the assumptions involved in the calculation of (target) molecular weights, there is a question as to whether target theory is applicable in cases where the target is partially nonspecific, as is the case, for example, if a small specific (marker) region is immersed in a larger, nonspecific matrix required for absorption. In such situations, even if there is a fixed requisite matrix size, the actual target size varies, depending on the size of the intact region of each molecule and the position of the marker within it. Furthermore, the boundaries of the matrix itself may be indefinite rather than fixed, so that the property associated with the matrix changes proportionally as the matrix size decreases, and single-hit target theory is inapplicable. First, however, one must decide what the nature of the target is.

Since in the studies under discussion transforming activity was always assayed in the region of linear response to concentration, we can consider the general equation for transformation (eq. [10]) in its limiting form:

$$\text{No. trans.} \propto M_n\,[D]\left(\left\{\overline{\alpha[B_0]k_mK_m}\right\}_I + \left\{\overline{\alpha[B_0]k_mK_m}\right\}_{II}\right)\left(\frac{NX}{M_n}\right). \qquad (13)$$

If the results are compared for a constant weight of DNA, $M_n\,[D]$ is constant. Of the remaining variables, we have shown that k_{mI}, α, possibly $[B_0]_I$, $[B_0]_{II}$ and k_{mII} and probably K_{mI} are functions of molecular weight. Almost certainly the variables depend also upon molecular shape, and α may depend on the location

of the marker within its molecule. Furthermore, neither absorption nor genetic incorporation is an all-or-nothing affair which decreases suddenly from a constant value to zero. Thus there is no discrete minimum functional size but a more or less continuous spectrum of target sizes, each of which is transmuted by a hit into a different, less efficacious, but still extant target.

An additional complication arises in radiation work, where three types of damage are possible: double-chain cleavage, single-chain cleavage, and secondary-bond cleavage (which results in shape change). The different steps in transformation may be affected differently by each of these, thus making the nature of the target a function of the relative frequencies of the three cleavages.

Under the conditions outlined above, physically meaningful "target" sizes cannot be expected. Furthermore, it is no surprise (and probably of little significance) that multisloped inactivation curves are frequently obtained. The disappearance of Type I sites, for instance, is probably one cause (although the rate at which this occurs need not be the same when materials of different polydispersity are used and when assays are carried out under different conditions of DNA concentration), superimposed on a myriad of others arising from a multiplicity of changing targets.

Degradation studies using ultraviolet light, DNase, and so forth are subject to many of the same criticisms. The problem of several types of damage and of a number of variables, each of which may depend on the extent of each type of damage, make the physical interpretation of activity-dose results impossible in the absence of more specific experimental data. The differential "stabilities" of certain markers toward various agents[19] face the same interpretive problems: rather than the markers themselves differing intrinsically in stability, their probabilities of incorporation into the genome may be affected differentially by the molecular damage caused by the inactivating agent.

SUMMARY

The activity of a sample of DNA depends on its ability to be absorbed on the surface of the bacterium, absorbed through the membrane, and incorporated into the genome, as well as on the number of markers which it contains. An equation has been derived which relates the rate of destruction of markers to their size, and this, combined with the kinetic equation for transformation, gives the transforming activity as a function of marker size. The latter can be evaluated by cleaving the DNA molecules and following the loss in activity, but first it is necessary to determine the molecular-weight dependence of the adsorption (binding) constant, of the absorption rate constant, and of the probability of incorporation into the genome.

Simultaneous absorption and transformation measurements have been carried out with quadruply marked DNA degraded by mechanical shear. Information and conclusions bearing on the above variables and on the pneumococcal surface have been presented and are discussed quantitatively in terms of the equations developed for transformation, absorption, and marker destruction.

The salient points are: (1) a large part, although not necessarily all, of the observed loss in transforming activity can be attributed to decreased absorption of DNA; (2) the size of the genetic marker is small, possibly of the order of several nucleotides; (3) there are probably two types of absorption site on the pneumo-

coccal surface, one of which absorbs only DNA of molecular weight greater than about 2.2×10^6; (4) a constant number of mols rather than a constant weight of DNA is probably bound by each type of site at saturation; (5) the molar binding constant for DNA by bacterial sites is constant in the range studied below $M_n = 2.2 \times 10^6$; (6) the probability of incorporation of each marker into the bacterial genome has a different dependence on molecular weight.

Results obtained with other methods for degrading DNA are discussed in terms of the variables mentioned above and the applicability of target theory. It is concluded that the physical interpretation of such results is far more complex than has hitherto been realized.

APPENDIX

If T_{0i} is the number of nucleotide pairs in each of the ith molecules, n_i is the number of molecules having T_{0i} pairs, and P is the number of nucleotide pairs in a marker, then the number of markers in the ith fraction is proportional to $n_i(T_{0i} - P + 1)$ and the total number of markers is proportional to $\Sigma n_i(T_{0i} - P + 1)$; $[n_i(T_{0i} - P + 1)]/[\Sigma n_i(T_{0i} - P + 1)]$ of the markers reside in the ith fraction (provided that $T_{0i} \geqslant P$). Every fracture of an ith molecule has a probability proportional to $(P - 1)/(T_{0i} - P + 1)$ of destroying a marker. When the damage to the molecule consists only of molecular cleavage, the number of fractures is $(T_{0i}/T_{fi}) - 1$, where T_{fi} is the average number of nucleotide pairs in the molecules obtained from cleavage of an ith molecule. Then the total fraction of all the markers destroyed by cleavage is

$$\sum \left(\frac{T_{0i}}{T_{fi}} - 1\right)\left(\frac{P - 1}{T_{0i} - P + 1}\right)\left(\frac{n_i(T_{0i} - P + 1)}{\Sigma\, n_i(T_{0i} - P + 1)}\right) = (P - 1)\left(\frac{\sum n_i \frac{T_{0i}}{T_{fi}} - \Sigma n_i}{\Sigma n_i T_{0i} - P\Sigma n_i + \Sigma n_i)}\right).$$

Substituting

$$W/G = P, \qquad n = \Sigma n_i, \qquad n\bar{M}_0/G = \Sigma n_i T_{0i}, \qquad \text{and}^{20} \quad \frac{n\bar{M}_0}{\bar{M}_f} = \sum \frac{n_i T_{0i}}{T_{fi}}$$

(where G is the average weight of one nucleotide pair [646], W is the weight of one marker, $\bar{M}_0$ is the number-average molecular weight of the starting material, n_{fi} is the number of product molecules obtained from the ith fraction, and $\bar{M}_f$ is the number-average molecular weight of the product) gives

$$\frac{(W - G)\left(\frac{\bar{M}_0}{\bar{M}_f} - 1\right)}{\bar{M}_0 - W + G}$$

for the fraction of markers destroyed. The fraction of markers remaining,

$$\left(\frac{NX}{M_n}\right)_f \Big/ \left(\frac{NX}{M_n}\right)_0,$$

is 1 less the above quantity; rearrangement and substitution of M_n (the measured number-average weight) = $\bar{M}_f$ gives equation (4),

$$\left(\frac{NX}{M_n}\right)\Big/\left(\frac{NX}{M_n}\right)_0 = \left[-\frac{\bar{M}_0(W-G)}{\bar{M}_0 - W + G}\right]\frac{1}{M_n} + \left[\frac{\bar{M}_0}{\bar{M}_0 - W + G}\right].$$

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[10] Under the usual assay conditions (see n. 9) excess time is allowed for all steps in the transformation process except the initial ones, i.e., the absorption of DNA.

[11] This expression is generally valid for any method of random cleavage, provided that no other damage is done to the molecule. For nonrandom cutting, however, it necessitates the assumption that the markers are randomly distributed in the starting material. This assumption is reasonable for fairly low-molecular-weight preparations, since DNA apparently undergoes random degradation during preparation.

[12] $\bar{M}_{abs} = \dfrac{\overline{Mk_{m+c}K_{m+c}}}{\overline{k_{m+c}K_{m+c}}}$; a is true when $(k_{m+c}K_{m+c})$ is a nonlinear function of molecular weight.

[13] This is, of course, true for both types of binding; $(k_{(m+c)_i})_{II}$ is therefore proportional to $1/M_i$ for the case of molecular binding, or constant for the case of unit binding.

[14] Molecular-weight distributions were constructed (see "Materials and Methods") for two DNA samples (M_n = 1.0 and 1.5 $\times$ 10^6) having activities and absorptions on the flat parts of the corresponding curves. The fraction (m_i) of the markers located in each molecular-weight fraction was calculated (see Appendix) assuming various values for W, the marker size. Then for each DNA sample, $\sum m_i \frac{1}{\bar{M}_i} = \left(\overline{\frac{1}{M_m}}\right)$. It was found that when M_n decreased, $\dfrac{1}{M_n\left(\overline{\frac{1}{M_m}}\right)}$ increased. Thus the right side of equation (12) increases when α is constant, and the increase would be accentuated only if α were to decrease. Since for fairly large markers the left side of equation (12) simultaneously decreases, such a situation is inconsistent with the experimental results (expressed by equation [12]). It is difficult to imagine that α could increase with decreasing molecular weight; but, even if this were the case, it would be fortuitous and therefore extremely unlikely that changes in α should just balance those in the other (totally unrelated) quantities. However, when W is very small, $\dfrac{1}{M_n\left(\overline{\frac{1}{M_m}}\right)}$ increases only slightly (i.e., less than the experimental error in the

transformation curve). In this case NX/M_n is constant, and, with α constant, this situation is consistent with equation (12).

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[16] For type II sites, a unit basis is unlikely because the unit binding constant (K/number of units per molecule) increases as the molecular weight decreases, whereas one would expect approximate constancy if there were a site for each unit. However, the *molar* binding constant (K) for type II sites is independent of molecular weight; the simplest assumption consistent with this result is that a part of the molecule is bound to the surface while another part resides in the surrounding medium. Thus, as the molecular weight decreases, less of the molecule protrudes into the medium. Type I sites appear to be large enough to encompass an entire molecule, since the molar rate of absorption and possibly the binding constant decrease with molecular weight.

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[20]
$$n_{fi} = n_i \frac{T_{0i}}{T_{fi}}; \quad \frac{\bar{M}_f}{G} = \frac{\sum n_i \frac{T_{0i}}{T_{fi}} T_{fi}}{\sum n_i \frac{T_{0i}}{T_{fi}}}; \quad \therefore \sum n_i \frac{T_{0i}}{T_{fi}} = \frac{\sum n_i \frac{M_{0i}}{G}}{\frac{\bar{M}_f}{G}} = \frac{n\bar{M}_0}{\bar{M}_f}.$$

SIZE OF PORES AND THEIR CONTENTS IN SIEVE ELEMENTS OF DICOTYLEDONS

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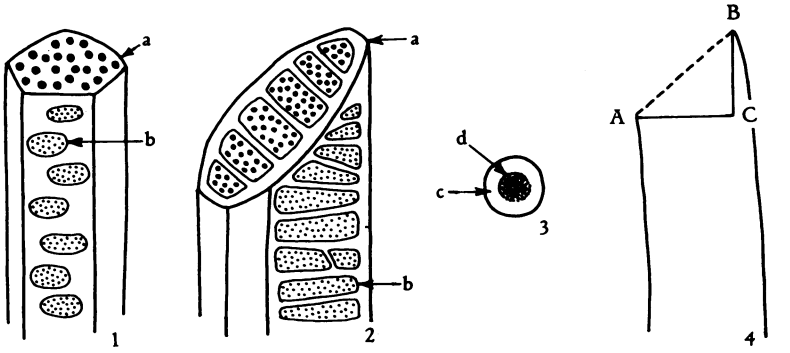
Sieve elements—the specialized conducting cells in the phloem tissue—have characteristic wall structures called "sieve areas." These constitute—at least in a highly differentiated state—clusters of pores (Figs. 1, 2, 5, 10) through which the protoplasts of vertically or laterally contiguous cells are interjoined. The



FIGS. 1–4.—Parts of sieve elements, one (Fig. 1) with a simple transverse sieve plate (*a*), the other (Fig. 2) with a compound inclined sieve plate (*a*). Both show also lateral sieve areas (Figs. 1, 2, at *b*). Fig. 3, transection of a sieve area pore with callose (*c*) inclosing the connecting strand (*d*). Fig. 4 is a diagram of part of a sieve element indicating length of sieve plate at AB and diameter of cell at sieve plate at AC.