

**A Statistical Analysis of Deoxyribonucleic Acid Distribution in Density  
Gradient Centrifugation**



Noboru Sueoka

*Proceedings of the National Academy of Sciences of the United States of America*,  
Volume 45, Issue 10 (Oct. 15, 1959), 1480-1490.

---

Your use of the JSTOR database indicates your acceptance of JSTOR's Terms and Conditions of Use. A copy of JSTOR's Terms and Conditions of Use is available at <http://www.jstor.org/about/terms.html>, by contacting JSTOR at [jstor-info@umich.edu](mailto:jstor-info@umich.edu), or by calling JSTOR at (888)388-3574, (734)998-9101 or (FAX) (734)998-9113. No part of a JSTOR transmission may be copied, downloaded, stored, further transmitted, transferred, distributed, altered, or otherwise used, in any form or by any means, except: (1) one stored electronic and one paper copy of any article solely for your personal, non-commercial use, or (2) with prior written permission of JSTOR and the publisher of the article or other text.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

*Proceedings of the National Academy of Sciences of the United States of America* is published by National Academy of Sciences. Please contact the publisher for further permissions regarding the use of this work. Publisher contact information may be obtained at <http://www.jstor.org/journals/nas.html>.

---

*Proceedings of the National Academy of Sciences of the United States of America*  
©1959 National Academy of Sciences

JSTOR and the JSTOR logo are trademarks of JSTOR, and are Registered in the U.S. Patent and Trademark Office. For more information on JSTOR contact [jstor-info@umich.edu](mailto:jstor-info@umich.edu).

©2001 JSTOR

<http://www.jstor.org/>  
Tue Apr 10 14:31:34 2001

‡ Porphyrins are endowed, in general, with a very great resonance energy which is responsible for the relative stability of these molecules. Thus, the resonance energy of the protoporphyrine IV is 10,570  $\beta$ , which, with the usual value adopted for  $\beta$  in this type of calculation, namely  $\beta = 20$  kcal/mole, represents about 210 kcal/mole. Although the rupture of the cyclic macro-ring represents a loss of a part of the resonance energy, this energy is still appreciable in the bile pigments: e.g., the resonance energy of biliverdin is 9,851  $\beta$  which is nearly 200 kcal/mole.

<sup>1</sup> For more details about these transformations see, e.g., R. Lemberg and J. W. Legge, "Haematin Compounds and Bile Pigments" (New York: Interscience Publishers, 1949), or C. H. Gray, "The Bile Pigments" (London: Methuen Ltd., 1953).

<sup>2</sup> For a general description of the method see, e.g., B. Pullman and A. Pullman. "Le théories électronique de la chimie organique" (Paris: Masson Ed., 1952).

<sup>3</sup> Pullman, B., and A. Pullman, these PROCEEDINGS, 44, 1197 (1958).

<sup>4</sup> Pullman, B., and A. Pullman, these PROCEEDINGS, 45, 136 (1959).

<sup>5</sup> E.g., H. C. Longuet-Higgins, C. Rector, and J. R. Platt, *J. Chem. Phys.* 18, 1174 (1959); T. Nakajima and H. Kon, *J. Chem. Phys.* 20, 750 (1952); S. L. Matlow, *J. Chem. Phys.* 23, 673 (1955); G. R. Seely, *J. Chem. Phys.* 27, 125 (1957); H. Kobayashi, *J. Chem. Phys.* 30, 1373 (1959).

<sup>6</sup> Pullman, B., and A. Pullman, *Biochim. et Biophys. Acta* (in press). See also G. Karreman, I. Isenberg, and A. Szent-Györgyi, *Science* (in press).

## A STATISTICAL ANALYSIS OF DEOXYRIBONUCLEIC ACID DISTRIBUTION IN DENSITY GRADIENT CENTRIFUGATION

BY NOBORU SUEOKA

THE BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY

Communicated by Paul Doty, August 25, 1959

Meselson, Stahl, and Vinograd (1957)<sup>1</sup> have established a powerful technique for providing information on molecular weight, density, and their heterogeneities in one operation. A brief description of the way the technique is applied to deoxyribonucleic acid (DNA) is as follows; two to three micrograms of DNA in a 7.7 molal cesium chloride solution of density 1.7 are centrifuged in the SPINCO Model E analytical ultracentrifuge. After an equilibrated density gradient of cesium chloride is established, DNA molecules converge to a position in the gradient corresponding to its density and form a narrow band. Theoretically it has been shown that in the absence of heterogeneities of both density and molecular weight the distribution of DNA molecules is Gaussian with a standard deviation ( $\sigma$ ) which is a function of the molecular weight of the DNA sample used. In the presence of molecular weight heterogeneity, both number and weight average molecular weights can be derived from the band profile.<sup>1</sup>

A DNA sample can have, however, both molecular weight and density heterogeneities. Recently it has been shown that the molecular density of DNA is related to its base composition.<sup>2, 3</sup> It has been also shown that heat denaturation<sup>4, 2</sup> and incorporation of either 5-bromouracil,<sup>1</sup> or heavy isotopes of nitrogen<sup>4, 5</sup> and carbon<sup>6</sup> change the density.

In order to analyze the experimental distribution of DNA in the band when both molecular weight and density heterogeneities exist, a general theory is presented and its applications to sonicated molecules of calf thymus and pneumococcus DNA are described.

*Scales and Their Transformations.*—Because the density gradient is essentially constant within the band range and it has been established that there is a linear relationship between the density and guanine-cytosine content of DNA molecules, three linear scales exist for the discussion of our problem and provide the following linear transformations among them. These are: (1) distance from the center of rotation in centimeter,  $r$ , (2) effective density,  $\rho$ , and (3) the guanine-cytosine fraction in DNA molecules,  $p$ ,

$$\rho = \left( \frac{d\rho}{dr} \right)_{\bar{r}} (r - \bar{r}) + \bar{\rho} \quad (\text{reference 1})$$

$$\rho = 0.103p + 1.662 \quad (\text{reference 2})$$

$$p = 9.71 \left( \frac{d\rho}{dr} \right)_{\bar{r}} (r - \bar{r}) + \bar{p}$$

where  $\bar{r}$ ,  $\bar{\rho}$  and  $\bar{p}$  are the means of the distribution in  $r$ -,  $\rho$ - and  $p$ -scales, respectively, and  $(d\rho/dr)_{\bar{r}}$  is the density gradient at  $\bar{r}$  which can be calculated by equation (38) of reference (7).

The  $p$ - and  $\rho$ -scales are more convenient than the  $r$ -scale, for the statistics on these scales are independent of the conditions applied in particular experiments and are therefore directly comparable. A special advantage of the  $p$ -scale is obvious in the case of fragmentation of DNA, which will be discussed later. This scale has also an advantage that the chemically determined guanine-cytosine content can be used as the mean ( $\bar{p}$ ). Among linearly related scales transformations of variance ( $\sigma^2$ ) from one scale to another are generally made by means of the relation:

$$\sigma^2(ax + b) = a^2\sigma^2(x)$$

*Analysis of Native DNA Distribution.*—In general on the  $x$ -scale we define the actual distribution of native DNA in a band by a function,  $T_N(x)$ ; similarly the DNA distribution in the absence of thermal agitation (that is, the real density distribution of the molecules) by  $D_N(X)$ ; the distribution caused solely by thermal agitation of the molecules having the value  $X$  of  $D_N(X)$  by  $B_N(X, x)$ . Here  $x$  and  $X$  are on the same scale and  $X$  is the mean of  $B_N(X, x)$ . The subscript  $N$  refers to native DNA. Each function is defined as follows:

$$\begin{aligned} \int_R T_N(x) dx &= C \\ \int_R D_N(X) dX &= C \\ \int_R B_N(X, x) dx &= 1 \end{aligned}$$

where  $R$  indicates that the integration covers the whole range of the distribution, and  $C$  is the total amount of DNA in the band measured by ultraviolet absorption (weight concentration). Now the actual distribution  $T_N(x)$  is

$$T_N(x) = \int_R D_N(X) B_N(X, x) dX, \quad (1)$$

and the variance of this function is by definition

$$\sigma_{TN}^2 = \frac{1}{C} \int_R (x - \bar{x})^2 T_N(x) dx,$$

where  $\bar{x}$  is the mean of the actual distribution in  $x$ -scale. Combining equation (1) and the above we get

$$\sigma_{TN}^2 = \frac{1}{C} \iint_R (x - \bar{x})^2 D_N(X) B_N(X, x) dX dx. \quad (2)$$

As we can put

$$(x - \bar{x})^2 = (x - X)^2 + (X - \bar{x})^2 + 2(x - X)(X - \bar{x}),$$

$$\begin{aligned} \sigma_{TN}^2 = & \frac{1}{C} \iint_R (x - X)^2 D_N(X) B_N(X, x) dX dx + \\ & \frac{1}{C} \iint_R (X - \bar{x})^2 D_N(X) B_N(X, x) dX dx + \\ & \frac{2}{C} \iint_R (x - X)(X - \bar{x}) D_N(X) B_N(X, x) dX dx \quad (3) \end{aligned}$$

The first term of equation (3) becomes

$$\frac{1}{C} \int_R D_N(X) \left[ \int_R (x - X)^2 B_N(X, x) dx \right] dX = \frac{1}{C} \int_R D_N(X) \sigma_{BN_X}^2 dX = \sigma_{BN}^2 \quad (4)$$

where  $\sigma_{BN_X}^2$  is the variance of  $B_N(X, x)$  and  $\sigma_{BN}^2$  is the mean variance of  $B_N$  functions. The second term similarly becomes

$$\frac{1}{C} \int_R (X - \bar{x})^2 D_N(X) \left[ \int B_N(X, x) dx \right] dX = \frac{1}{C} \int_R (X - \bar{x})^2 D_N(X) dX = \sigma_{DN}^2$$

where  $\sigma_{DN}^2$  is the variance of  $D_N(X)$  or the variance due to density heterogeneity. The third term is zero. Thus,

$$\sigma_{TN}^2 = \sigma_{BN}^2 + \sigma_{DN}^2. \quad (5)$$

Here  $\sigma_{BN}^2$  should be calculated by equation (9) of reference (1) using a number average molecular weight determined by independent methods.<sup>8</sup> The total variance  $\sigma_{TN}^2$  is calculated by numerical integration of the observed distribution with Sheppard's correction. Equation (5) shows that the total variance ( $\sigma_{TN}^2$ ) calculated from the band profile is the sum of a variance ( $\sigma_{BN}^2$ ) caused by Brownian motion of molecules which is molecular weight dependent and a variance ( $\sigma_{DN}^2$ ) due to density heterogeneity.

The number average molecular weight ( $M_N$ ) of native DNA can be obtained from a sedimentation profile in the following way. First the sedimentation constant ( $s_{20, w}^{50}$ ) is obtained from the transition of the 50 per cent points of sedimentation profile along with the time. The weight average molecular weight ( $M_W$ ) can be obtained from the empirical equation<sup>10</sup>

$$s_{20, w}^{50} = 0.063 M_W^{0.37}. \quad (6)$$

It should be noted that the value 0.063 is valid only for  $s_{20, w}^{50}$ . The distribution of sedimentation coefficients ( $s_{20, w}$ ) is obtained by the procedure of Schumaker and Schachman.<sup>9</sup> The ratio, weight average to number average molecular weight ( $M_W/M_N$ ) can be calculated by

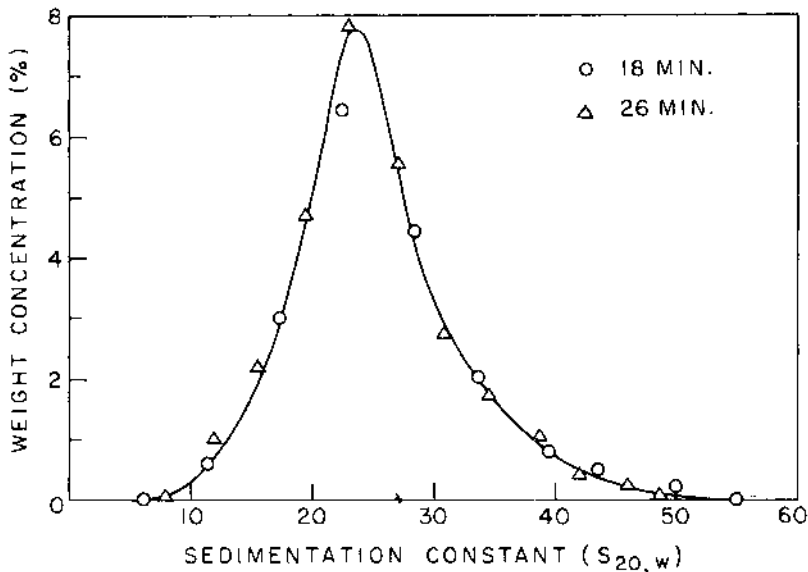


Fig. 1.—Distribution of sedimentation coefficients of native calf thymous DNA calculated from sedimentation profile. The sedimentation pattern was photographed with Kodak X-Ray Film with ultraviolet light. The initial concentration of DNA is 15  $\mu\text{g}$  per ml. The cell depth is 3 cm and the total optical density at 260  $m\mu$  at the plateau of the picture is less than 1. Circles were calculated from 18-min picture and triangles from 26-min picture. The tracings of the photographs were made by Joyce double beam microdensitometer and the effective slit width is 0.064 mm. Points for the two different times agree very well indicating the negligible effect of diffusion and convection during the centrifugation.

$$M_w/M_N \simeq \frac{(\sum_i y_i s_i^{2.7})(\sum_i y_i s_i^{-2.7})}{(\sum_i y_i)^2} \quad (7)$$

(see appendix)

where  $s_i$  is a sedimentation coefficient and  $y_i$  is the incremental weight fraction of  $s_i$ . From the ratio and the  $M_w$ , the number average molecular weight can be obtained.

The transformation of the  $s_{20,w}$ -distribution to the molecular weight distribution is not necessary for the calculation of  $M_N$ . It is possible, however, to make such transformation, which may visualize the extent of the molecular weight heterogeneity. The necessary equations are

$$M_i = \frac{M_w s_i^{2.7}}{\beta} \quad (8)$$

$$Y_i = \frac{0.37 s_i}{M_i} y_i \quad (9)$$

where

$$\beta = \frac{\sum_i s_i^{2.7} y_i}{\sum_i y_i} \quad (10)$$

(see appendix)

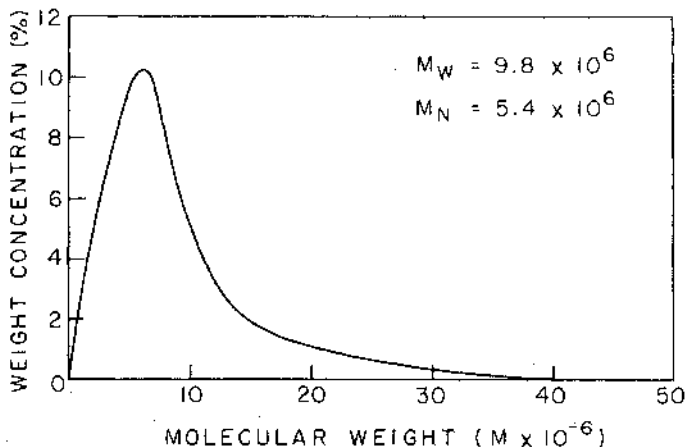


FIG. 2.—Distribution of the molecular weight of calf thymus DNA calculated from Fig. 1. The ordinate is the weight fraction in percentage. The transformation from  $s_{20,w}$  to  $M$  was made by equations (8) and (9) in the text using the smoothed curve of Fig. 1. The weight and number average molecular weights were calculated by the methods described in the text.

The  $M_i$  is the molecular weight corresponding to  $s_i$ , and  $Y_i$  is the weight fraction of  $M_i$ . The calculation by equations (7)–(10) are greatly facilitated by making the table of  $s^{2.7}$  and  $s^{-2.7}$  for discrete values of  $s$  from 1 to 100. Figures 1 and 2 show the distribution of  $s_{20,w}$  and molecular weight ( $M$ ) of a native calf thymus DNA. The ratios  $M_W$  to  $M_N$  calculated for calf thymus and pneumococcus DNA are 1.8 and 1.2, respectively.

In general the extent of density heterogeneity may be conveniently expressed as percentage by,

$$H_D = \frac{\sigma_T^2 - \sigma_B^2}{\sigma_T^2} \times 100 \quad (11)$$

where  $\sigma_T^2$  is the total variance and  $\sigma_B^2$  is the variance due to thermal agitation. It may be noted that the relative magnitude of the variances in equations (5), (11), and (12) are independent of the scale used.

As examples, analyses on native calf thymus and pneumococcus DNA will be described. Sedimentation coefficient, molecular weights, and ratios ( $M_W/M_N$ ) are given in Table 1. Tracings of ultraviolet absorption photographs of DNA

TABLE 1  
SEDIMENTATION COEFFICIENTS AND MOLECULAR WEIGHTS OF CALF THYMUS AND PNEUMOCOCCUS DNA†

Source of DNA	$s_{20,w}^0$	$M_W^*$	$M_W/M_N$	$M_N^*$
Calf thymus	24.4	$9.8 \times 10^6$	1.8	$5.4 \times 10^6$
Pneumococcus	24.9	$10.4 \times 10^6$	1.2	$8.7 \times 10^6$

\* Sodium salt of DNA.

† The calf thymus DNA has been prepared by Simmon's detergent method,<sup>10</sup> and the pneumococcus DNA by a chloroform-octanol method,<sup>21</sup> from *Diplococcus pneumoniae* strain R36A.

TABLE 2  
 VARIANCES AND DENSITY HETEROGENEITY INDICES ( $H_D$ ) OF CALF THYMUS  
 AND PNEUMOCOCCUS DNA

Source of DNA	Variances ( $\times 10^4$ )*			$H_D$ , %
	$\sigma_{TN}^2(p)$	$\sigma_{BN}^2(p)$	$\sigma_{DN}^2(p)$	
Calf thymus	27.8	4.8	23.0	83
Pneumococcus	6.9	3.0	3.9	57

\* Variances are given in the  $p$ -scale.  $\sigma_{BN}^2(p)$  was calculated from  $1.33 \times M_N$  which is the number average molecular weight of cesium salt of DNA.

bands are shown in Figure 3 and the calculated variances in the  $p$ -scale are summarized in Table 2. The actual (total) variance of calf thymus DNA is four times as large as that of pneumococcus DNA. It is noted that the main part of the difference comes from the large density heterogeneity of the calf thymus DNA.

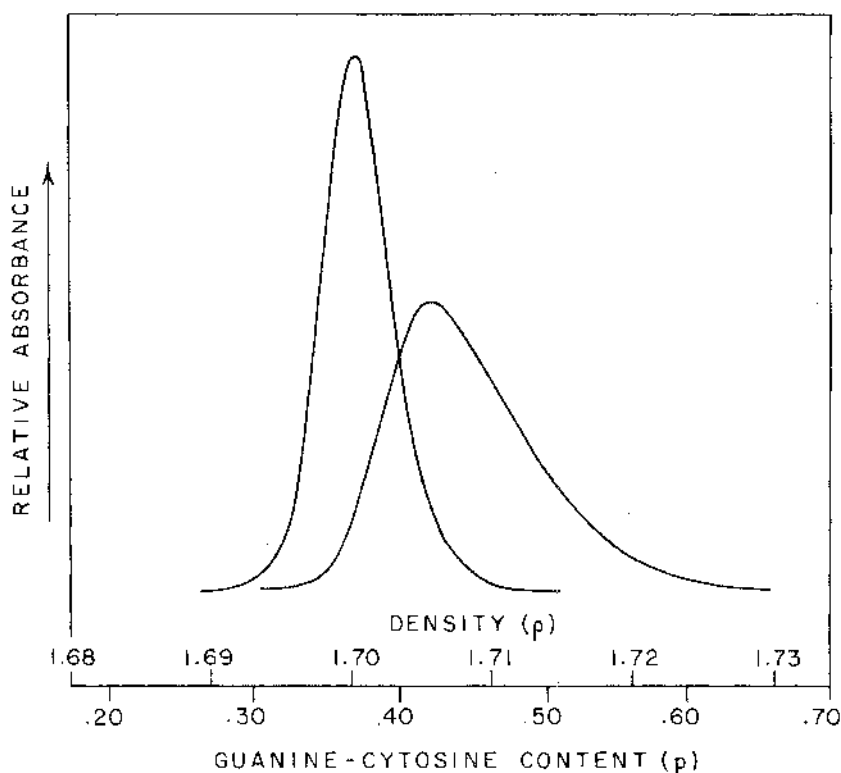


FIG. 3.—Microdensitometer tracings of the ultraviolet absorption photographs of equilibrated calf thymus and pneumococcus DNA in density gradient centrifugation. Both DNA samples were purified by chloroform octanol method, and separately centrifuged at 44,770 rpm for more than 50 hr. Equilibrium was checked by comparing the band profiles at about 12-hr intervals. Tracings were made using a Joyce microdensitometer with an effective slit width (in the cell dimension) of 0.013 mm. The curves have been smoothed to eliminate the background noise normally found in tracings. The area under the curves has been made equal for better visual comparison. The  $\bar{p}$  values for calf thymus and pneumococcus DNA adopted in this figure are 0.44 and 0.37, respectively.

*Analysis of Fragmented DNA.*—In this section we will deal with the analysis of fragmented molecules, where each molecule is split into an average of  $\nu$  pieces.

*General case:* We designate the actual distribution of fragmented DNA in the

density gradient field by  $T_F(x)$ . The intrinsic density distributions of the native and the fragmented DNA in the absence of thermal agitation are given by  $D_N(X)$  and  $D_F(X, X')$ , respectively. Here  $D_F$  is the distribution function of density of the fragments originated from the native molecules which had the density  $X$ . The thermal distribution of the fragmented molecules having the density  $X'$  of  $D_F(X, X')$ , is expressed by  $B_F(X, X', x)$ . The variance relationship of these functions is obtained by operations similar to those described before. Thus,

$$\sigma_{TF}^2 = \sigma_{BF}^2 + \sigma_{DN}^2 + \sigma_{DF}^2 \quad (12)$$

or noting  $\sigma_B^2 \propto M^{-1}$ ,

$$\sigma_{TF}^2 = \nu \sigma_{BN}^2 + \sigma_{DN}^2 + \sigma_{DF}^2 \quad (13)$$

Here  $\sigma_{TF}^2$  is the variance of  $T_F$  and  $\sigma_{BF}^2$  and  $\sigma_{DF}^2$  are the mean variances of  $B_F$ 's and  $D_F$ 's, respectively. The total variance,  $\sigma_{TF}^2$ , is obtained like  $\sigma_{TN}^2$  from the band profile, and  $\sigma_{BF}^2$  from number average molecular weight of the fragmented molecules. The value of  $\sigma_{DN}^2$  is obtained from the analysis of native DNA. The residual variance is an estimate of  $\sigma_{DF}^2$ . This estimate, however, is subject to accumulated errors of the estimates of other variances. Independent estimation of  $\sigma_{DF}^2$  is, therefore, desirable, which will be discussed for sonicated and heated samples in the following sections.

*Application for sonicated molecules:* Upon sonication, each DNA molecule is cut into pieces without separating the two chains of the Watson-Crick helix.<sup>10</sup> If we assume a random distribution of guanine-cytosine and adenine-thymine pairs within each molecule and that  $\sigma_{DN}^2$  is totally due to base composition heterogeneity, we can calculate an expected value of  $\sigma_{DF}^2$  as follows: taking the  $p$ -scale, the variance newly arising by sonication of native molecules having guanine-cytosine content  $p$  is

$$\sigma'_{DFp^2}(p) = \frac{p(1-p)}{b},$$

where  $b$  is the average number of base pairs in sonicated molecules. For the entire distribution, however, the expected variance is

$$\begin{aligned} \sigma'_{DF^2}(p) &= \frac{1}{C} \int_R \sigma'_{DFp^2}(p) D_N(p) dp \\ &= \frac{1}{Cb} \int_R p(1-p) D_N(p) dp \\ &= \frac{1}{b} [\bar{p}(1-\bar{p}) - \sigma_{DN}^2(p)] \end{aligned} \quad (14)$$

where  $\bar{p}$  is the average guanine-cytosine content of the DNA sample. Here upon sonication it is assumed that  $\bar{p}$  remains as the average of the sonicated sample.<sup>22</sup> This provides a possibility to test the randomness of the pair distribution within the molecule, by comparing  $\sigma_{TN}^2$  with the sum of the three variances ( $\sigma_{BF}^2$ ,  $\sigma_{DN}^2$  and  $\sigma'_{DF}^2$ ). If the distribution of two kinds of base pairs within the molecule is predominantly nonrandom, the sum is expected to be larger than  $\sigma_{TN}^2$ , while smaller

TABLE 3  
MOLECULAR WEIGHTS AND VARIANCES OF SONICATED CALF THYMUS AND PNEUMOCOCCUS DNA

Source of DNA	$s_{0,w}^0$	$M_w$	Variances ( $\times 10^4$ )*				$H_D, \%$
			$\sigma_{TF}^2(p)$	$\sigma_{BF}^2(p)$	$\sigma_{DN}^2(p)$	$\sigma'_{DF}^2(p)$	
Calf thymus	9.7	$0.83 \times 10^6$	79.8	61.5	23.0	2.6	23
Pneumococcus	12.5	$1.6 \times 10^6$	37.4	31.9	3.9	0.8	15
					87.1		
					36.6		

\* Variances are given in the  $p$ -scale.  $\sigma_{BF}^2(p)$  was calculated from the half of  $1.33 \times M_w$  assuming  $M_N$  is approximately half of  $M_w$ . The factor 1.33 is for converting sodium salt to cesium salt of DNA. The  $p$  values for calf thymus and pneumococcus DNA used in calculation of  $\sigma'_{DF}^2(p)$  are 0.44 and 0.37, respectively.

if a regular nonrandomness (e.g., a tendency toward alternation) is dominant. The analysis of sonicated samples of calf thymus and pneumococcus DNA<sup>11</sup> is summarized in Table 3. The ratios,  $M_w/M_N$ , calculated directly from sedimentation pattern are 2.5 for calf thymus 2.8 for pneumococcus. These values are, however, too large for small molecules because of the effect of diffusion. Since electron micrographs of sonicated calf thymus DNA give the ratio approximately two<sup>12</sup> in the present analysis the ratio two is used.

*Application for heated molecules:* We can apply equation (13) as such for heat denatured molecules. If we assume that two component chains of the double helix separate by heating, the variance equation (13) becomes,

$$\sigma_{TF}^2 = 2\sigma_{BN}^2 + \sigma_{DN}^2 + \sigma_{DF}^2 \quad (15)$$

Effective density of a heated DNA increases by a universal value,  $0.015^{2,4}$  so that the linear relationship between guanine-cytosine content and density maintains its slope, allowing to use the same scale for  $\sigma_{DF}^2$  as for the other terms. A theoretical estimation of  $\sigma_{DF}^2$  should be obtained from random deviation of guanine or cytosine from  $p/2$  and adenine or thymine from  $(1-p)/2$  among single chains. Unfortunately we do not have experimental data on the effective density difference between guanine and cytosine or adenine and thymine. The value,  $\sigma_{DF}^2$ , however, will be quite small because of the large number of nucleotides in the denatured molecule of the native DNA.

*Discussion.*—It has been shown<sup>13</sup> that if the macromolecular material examined by the density gradient centrifugation has a Gaussian distribution of the effective density, it will form a Gaussian band and thus appears to be homogeneous. The variance of the resulting distribution in this case is a simple sum of the two component variances, one for the effective density distribution and the other for thermal agitation. Consequently it has been pointed out that with the presence of density heterogeneity, the molecular weight estimated by this technique gives an underestimation. However, the two assumptions, homogeneous molecular weight and Gaussian distribution of effective density, are not satisfied in general. Furthermore, it has been shown that DNA from calf thymus, salmon sperm and mouse have skewed distributions.<sup>7,2</sup> The solution given in this paper is general and can be applied for the cases in which both density and molecular weight heterogeneities are present; it has no restrictions on the mode of their distributions.

It is apparent from earlier work<sup>2,16</sup> that the principal cause of density hetero-

TABLE 4

RANGE OF BASE COMPOSITION DISTRIBUTION IN GUANINE-CYTOSINE FRACTION OF CALF THYMUS AND PNEUMOCOCCUS DNA ESTIMATED FROM THREE DIFFERENT METHODS

Source of DNA	Density* Gradient Centrifugation [ $\bar{p} \pm 2\sigma_{DN}(\bar{p})$ ]	Hyperchromic† Effect by Heating ( $\bar{p} \pm 2\sigma$ )	Chemical‡ Fractionation
Calf thymus	0.34-0.54	0.33-0.55	$\left\{ \begin{array}{l} 0.40-0.48^{14} \\ 0.40-0.55^{15} \\ 0.34-0.52^{16} \end{array} \right.$
Pneumococcus	0.33-0.41	0.33-0.41	...

\* The  $\bar{p}$  values for calf thymus and pneumococcus adopted in this table are 0.44 and 0.37, respectively.

† See reference (18).

‡ Ranges were taken from the upper and lower extreme values of the references indicated.

genity in DNA is the compositional variation among the molecules of a given sample. The adequacy of this explanation can be checked to some extent by comparing the estimates of composition heterogeneity obtained by two other methods (fractionation and absorbance-temperature curves) with that obtained from the analysis of density gradient band profiles. The existence of such heterogeneity in base composition has been analyzed chemically by fractionation of native molecules of DNA of calf thymus,<sup>14-16</sup> pig liver,<sup>14</sup> sea urchins,<sup>14</sup> *E. coli*,<sup>16</sup> and  $T_{2r}$ ,<sup>16</sup> and  $T_6$ ,<sup>14</sup> phages. Another evidence came from hyperchromic effect of DNA by thermal inactivation.<sup>17, 18</sup> The range of the heterogeneity detected by these three methods in calf thymus and pneumococcus DNA is summarized in Table 4. These data agree fairly well and therefore we can conclude that at least most of the density heterogeneity is due to base composition variation among the molecules of calf thymus and pneumococcus DNA.

The molecular weight which is used to calculate the  $\sigma_B^2$  should be the number average molecular weight. The method of obtaining the number average molecular weight described in this paper is not exact but the approximation involved does not appear to be serious. This approximation is based on the fact that the exponent  $\alpha$  of equation (1') of the appendix is constant while the coefficient,  $K$ , is subject to small variation depending on the molecular weight distribution within the sample and on the deviation of the mean and the median of the sedimentation distribution. Experimental data at present do not appear to justify a more elaborate approach to the number average molecular weight. In addition the procedure used here is simple and generally applicable.

If there is no density and molecular weight heterogeneity present, the sonicated DNA (Table 3) should have more than nine times larger variance in calf thymus DNA and five times larger variance in pneumococcus DNA than those of the native molecules. The actual variance of the sonicate, however, is found to be only three times as large as the native variance in calf thymus DNA which indicates the presence of a large proportion of density heterogeneity. A fair agreement between the total variance and sum of three variances ( $\sigma_{BF}^2$ ,  $\sigma_{DN}^2$ ,  $\sigma'_{DF}^2$ ) in both calf thymus and pneumococcus DNA indicates that the distribution of adenine-thymine and guanine-cytosine pairs within molecule is not far from random in these cases.

Our analysis of variances has some relevance to the conclusions that have been reached concerning the halving of the molecular weight by heating in *E. coli* DNA,<sup>4</sup> while in calf thymus and salmon sperm DNA no change in band width was ob-

served. From equation (15) it is clear that even if the molecule does become half, the band width will not increase very much in case of the large relative values of  $\sigma_{DN}^2$  which remains in the equation. Therefore, it is quite possible that calf thymus and salmon sperm DNA reduce the molecular weight to half by heating but that the reduction is essentially undetectable from a comparison of the band width.

*Appendix.*—When sedimentation coefficient,  $s$ , and molecular weight,  $M$ , have the relation,

$$s = KM^\alpha \quad (1')$$

and the weight fractions for  $s$  and  $M$  are  $y$  and  $Y$ , respectively, the following relations are obtained.

$$yds = YdM \quad (2')$$

$$\begin{aligned} M_w &= \frac{\int_R YMdM}{\int_R YdM} = \frac{\int_R s^{1/\alpha} yds}{K^{1/\alpha} \int_R yds} \\ &\simeq \frac{\sum_i s_i^{1/\alpha} y_i}{K^{1/\alpha} \sum_i y_i} \quad (3') \end{aligned}$$

$$\begin{aligned} M_N &= \frac{\int_R YdM}{\int_R \frac{Y}{M} dM} = \frac{\int_R yds}{K^{1/\alpha} \int_R s^{-1/\alpha} yds} \\ &\simeq \frac{\sum_i y_i}{K^{1/\alpha} \sum_i s_i^{-1/\alpha} y_i} \quad (4') \end{aligned}$$

The  $R$  indicates the integration covers the whole range of the distributions. Therefore,

$$\begin{aligned} M_w/M_N &= \frac{(\int_R s^{1/\alpha} yds)(\int_R s^{-1/\alpha} yds)}{(\int_R yds)^2} \\ &\simeq \frac{(\sum_i s_i^{1/\alpha} y_i)(\sum_i s_i^{-1/\alpha} y_i)}{(\sum_i y_i)^2} \quad (5') \end{aligned}$$

Combining (1') and (3') and putting

$$\beta = \frac{\int_R s^{1/\alpha} yds}{\int_R yds} \simeq \frac{\sum_i s_i^{1/\alpha} y_i}{\sum_i y_i} \quad (6')$$

we get,

$$M_i = \frac{M_w s_i^{1/\alpha}}{\beta} \quad (7')$$

From (1') we get,

$$\frac{ds}{dM} = \alpha KM^{\alpha-1} = \alpha \frac{s}{M}$$

Combining this with (2') and (6') we get,

$$Y_i = \frac{\alpha s_i}{M_i} y_i \quad (8')$$

For  $\alpha = 0.37$  we obtain the equations (7), (8), (9), and (10) from (5'), (7'), (8'), and (6'), respectively. A transformation from  $s_{20, w}$ -distribution to  $M$ -distribution has been discussed by Williams and Saunders for integral form of distribution.<sup>19</sup>

The author is deeply indebted to Professor P. Doty for his constant encouragement and valuable suggestions. The pneumococcus DNA and its sedimentation picture were kindly supplied by Dr. J. Marmur, the sonicated calf thymus and the sonicated pneumococcus DNA were given by Mr. J. Eigner. Criticisms and suggestions by Dr. E. Freese and Dr. R. D. Luce are greatly acknowledged. The author is also indebted to Mr. J. Kucera for technical help. This work was supported by research grant E-1421 from the United States Public Health Service to Prof. R. P. Levine, from a National Science Foundation Grant, G7025 to Profs. R. P. Levine and J. D. Watson, and from a United States Public Health Service Grant C-2170 to Prof. P. Doty.

<sup>1</sup> Meselson, M., F. W. Stahl, and J. Vinograd, these PROCEEDINGS, **43**, 581 (1957).

<sup>2</sup> Sueoka, N., J. Marmur, and P. Doty, *Nature*, **183**, 1429 (1959).

<sup>3</sup> Rolfe, R., and M. Meselson, these PROCEEDINGS, **45**, 1039 (1959).

<sup>4</sup> Meselson, M., and F. W. Stahl, these PROCEEDINGS, **44**, 672 (1958).

<sup>5</sup> Sueoka, N., "Mitotic replication of deoxyribonucleic acid in *Chlamydomonas reinhardtii*" (in press).

<sup>6</sup> Davern, C., *J. Molecular Biology* (in press).

<sup>7</sup> Meselson, M., *Ph.D. Thesis*, California Institute of Technology (1958).

<sup>8</sup> It is clear from equation (4) of the present paper and equation (18) of reference (1) that  $\sigma_{DN}^2$  is inversely proportional to the number average molecular weight. The same DNA preparation should be used for the density gradient centrifugation and sedimentation coefficient determination.

<sup>9</sup> Schumaker, V. N., and H. K. Schachman, *Bioch. Biophys. Acta*, **23**, 628 (1957).

<sup>10</sup> Doty, P., B. B. McGill and S. A. Rice, these PROCEEDINGS, **44**, 432 (1958).

<sup>11</sup> For the conditions of sonication see M. Litt, J. Marmur, H. Ephrussi-Taylor and P. Doty, these PROCEEDINGS **44**, 144, 1958 and P. Doty, B. B. McGill, and S. A. Rice, these PROCEEDINGS, **44**, 432 (1958).

<sup>12</sup> Hall, C., and P. Doty, *J. Am. Chem. Soc.*, **80**, 1269 (1958).

<sup>13</sup> Baldwin, R. L., these PROCEEDINGS, **45**, 939 (1959).

<sup>14</sup> Crampton, C. F., R. Lipshitz and E. Chargaff, *J. Biol. Chem.*, **211**, 125 (1954).

<sup>15</sup> Bendich, A., H. B. Pahl, G. C. Korngold, H. S. Rosenkranz and J. R. Fresco, *J. Am. Chem. Soc.*, **80**, 3949 (1958).

<sup>16</sup> Brown, G. L., and A. V. Brown, *Symposia of the Society for Experimental Biology*, **12**, 6 (1958).

<sup>17</sup> Marmur, J., and P. Doty, *Nature*, **183**, 1427 (1959).

<sup>18</sup> Doty, P., J. Marmur and N. Sueoka, *Proceedings of Brookhaven Symposium* (in press).

<sup>19</sup> Williams, J. W., and W. Saunders, *J. Phys. Chem.*, **58**, 854 (1954).

<sup>20</sup> See Rice, S. A., and P. Doty, *J. Am. Chem. Soc.*, **79**, 3937 (1957).

<sup>21</sup> Marmur, J., in preparation.

<sup>22</sup> Note added in proof: It was found recently that sonication does not affect the density of either calf thymus or pneumococcus DNA.