

Cytopathology.—In cultures similar to those in which the development of virus was followed, stained preparations of the cells were made at intervals. In this manner, it has been possible to identify infected cells, to describe the sequence of the cytological changes and to estimate the degree of involvement of the culture. Cultures infected with influenza virus show an increase in granularity, with rounding, enlargement of cells followed by pyknosis of the nucleus, ending in cellular fragmentation and death. The cellular changes as a result of infection with adenovirus type 4 are typified by rounding, and fusion of cells into clusters with increased granularity. The cytopathogenic changes associated with measles are similar to those described by Katz *et al.*⁶ in chick embryo cultures. The involved cells exhibited a fusiform shape together with syncytia or multinuclear giant cells. Hemadsorption type I and II produce definite cytopathogenic changes characterized by focal areas of involvement with normal appearance of surrounding cells; the cytopathic effect of these two viruses are indistinguishable. The changes in cells infected by poliovirus consist of localized areas in which there is rounding, loss in cytoplasmic volume, increase in numbers of refractile cells, and pyknosis of the nucleus.

In summary, a chick kidney culture system has been developed in which a wide spectrum of viruses was propagated successfully. Factors have been described which influence the propagation of these agents. The growth of the viruses was always accompanied by cytopathic changes in the chick kidney cultures.

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⁴ Eagle, H., *J. Exp. Med.*, **102**, 37 (1955).

⁵ Dulbecco, R., and M. Vogt, *J. Exp. Med.*, **97**, 167 (1954).

⁶ Katz, S. L., M. V. Milovanovic, and J. F. Fnders, *Proc. Soc. Exp. Biol. and Med.*, **97**, 23 (1958)

THE RELATIVE HOMOGENEITY OF MICROBIAL DNA*

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Density-gradient centrifugation has revealed that the population of DNA molecules from *E. coli* is relatively homogeneous with respect to buoyant density in a solution of cesium chloride.¹ Because of the notably small apparent atomic volume of nitrogen in aqueous solutions of organic compounds² and because the guanine-cytosine base pair is more rich in nitrogen than is the adenine-thymine pair, it was considered that the density homogeneity among *E. coli* DNA molecules might reflect a high degree of homogeneity with respect to base composition. To investigate this possibility, an examination has been made of the relationship between buoyant density in cesium chloride solution and base composition of DNA from various sources.

We have measured the buoyant density of nine different bacterial DNA's ranging from 0.3 to 0.7 in mean molar fraction of guanine plus cytosine as determined by chromatographic analysis in one laboratory.³ The DNA from each bacterial species formed a single sharp band in the cesium chloride density gradient. The mean buoyant density of each banded bacterial DNA was determined with respect to the band formed by an aliquot of $C^{13}N^{15}$ *E. coli* DNA which served as a convenient density reference. Typical results are shown in Figure 1, which shows the bands

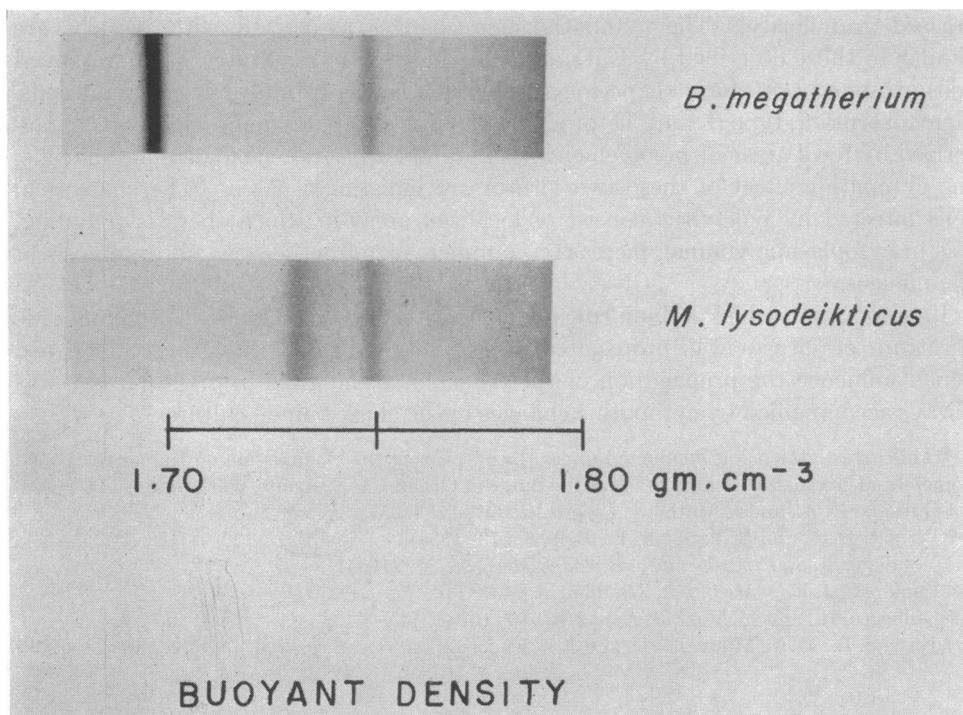


FIG. 1.—Ultraviolet absorption photographs showing bands of DNA from *B. megatherium* and *M. lysodeikticus* with reference bands of $C^{13}N^{15}$ *E. coli* DNA in 57.5 weight percent CsCl solution after 24 hours of centrifugation at 44,770 rpm. All bacteria were grown in broth at 37° C. to a titer of 2×10^8 and sedimented in the cold for ten minutes at $1800 \times g$. The pellet was resuspended in 0.4 ml. of a solution 0.3 *M* in NaCl, 0.01 *M* in sodium citrate, 0.015 *M* in versene, and 0.01 *M* in tris(hydroxymethyl)aminomethane buffered at pH 6.1. Bacteria were lysed with 0.1 ml. 10 percent sodium dodecyl sulfate or 5 percent sodium desoxycholate, and in some cases were exposed to 100 micrograms/ml lysozyme for 30 seconds before detergent was added. Conditions of ultracentrifugation of the lysates have been described previously.¹

formed by DNA from *B. megatherium* and from *M. lysodeikticus* and the accompanying reference bands of $C^{13}N^{15}$ *E. coli* DNA. As may be seen from Figure 2, a linear relationship⁴ given by the expression

$$\rho_{25^\circ} = 1.658 + 0.100 \text{ GC gm. cm}^{-3}.$$

is found between the mean buoyant density ρ at 25°C and the mean guanine-cytosine content GC defined as the molar ratio $\frac{G + C}{G + C + A + T}$. The observed value of $\frac{d\rho}{dGC}$ is considerably larger than the value 0.038 estimated from apparent atomic volumes in aqueous solution.²

Equation (1) was obtained from determinations of the mean composition and mean buoyant density of DNA from several species of bacteria but we may now apply it to the population of DNA molecules from a single species. In particular,

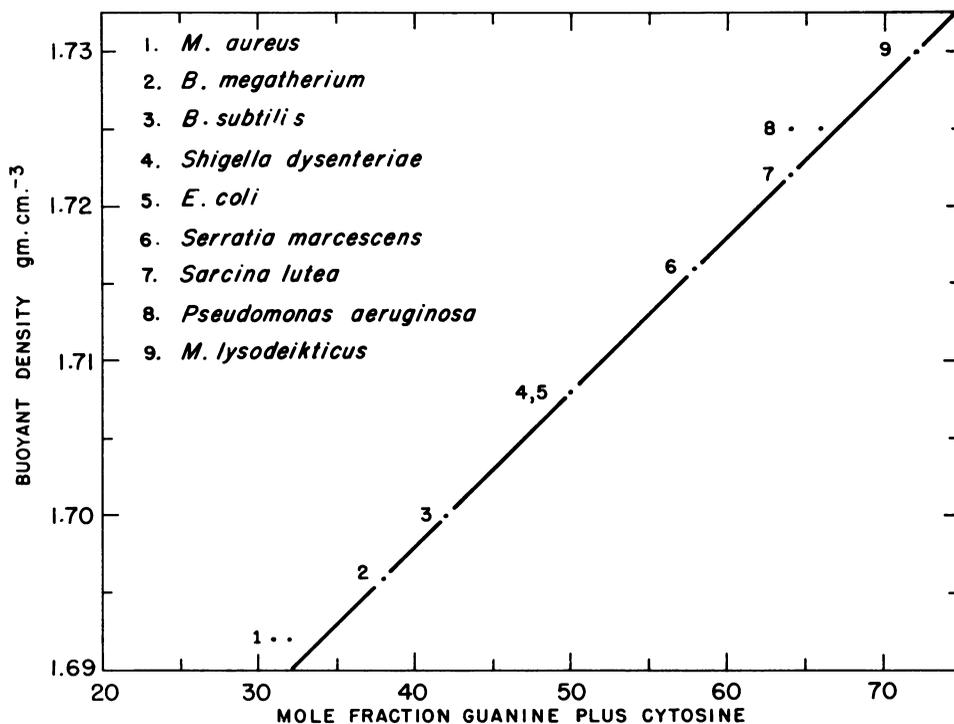


FIG. 2.—The density-composition relationship for DNA. Mean buoyant density at 25°C. (P_{25}) is plotted against mean molar fraction of guanine plus cytosine (GC) for the DNA from nine bacterial species. Where GC is variable among the studied strains of a given species, the range of variation is indicated by a pair of points. The base composition given for *Shigella dysenteriae* is that of *Shigella paradysenteriae*.

we shall compute for each species an upper bound on the standard deviation σ_{GC} of the distribution of the guanine-cytosine base pair over the population of DNA molecules. From equation (1) the upper bound on σ_{GC} is given as

$$\sigma_{GC \text{ max.}} = 10.0\sigma\rho$$

where $\sigma\rho$ is the standard deviation of the DNA distribution in the cesium chloride density gradient. This computation requires for its validity that no DNA band be seriously narrowed by intermolecular aggregation and that each band comprise a representative sample of the total DNA of the corresponding bacterial species. Aggregation is unlikely in view of the absence of aggregation in a cesium chloride density gradient between molecules of *E. coli* DNA differing only in their content of the heavy isotope N^{15} . It is very likely that the banded DNA is representative of the total DNA because of the close agreement between the mean GC content determined chromatographically and that calculated from buoyant density by means of equation (1). It should be pointed out that the actual value of σ_{GC} may lie considerably below the calculated upper bound because thermal motion of the DNA molecules contributes significantly to the bandwidth.

The DNA from each bacterial species investigated forms a band in the density gradient with σ_p in no case greater than 0.003 gm. cm⁻³. The corresponding upper bound on the standard deviation σ_{GC} of the molecular content of guanine plus cytosine is therefore in no case greater than 0.03.⁵ It is remarkable that the standard deviation of guanine-cytosine content within the molecular population of any one bacterial species covers less than one tenth of the range over which the mean guanine-cytosine content varies among the various species.

If we assume for purposes of discussion that much hereditary information is common to the various bacterial species and that DNA is a carrier of genetic information, then our finding that few, if any, DNA molecules possess compositions common to the various species argues against the conception that the complete nucleotide sequence could in principle be deduced from other hereditary characteristics according to a universal code. Instead, it may be that only certain features of the nucleotide sequence are genetically significant so that extensive modification of nucleotide composition need not result in any other genetic alteration. Or it may be that the detailed relation between nucleotide sequence and genetic specificity is itself a species characteristic.

As this investigation was being completed, we learned that Sueoka, Marmur, and Doty have independently arrived at a relation between DNA composition and buoyant density which is in good agreement with the relation reported here. We wish to thank these authors for communicating their findings to us before publication.

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‡ Contribution No. 2460.

¹ Meselson, M., and F. W. Stahl, these PROCEEDINGS, **44**, 671 (1958).

² Traube, J., *Samm. Chem. u. Chem.-Tech. Vorträge*, **4**, 255 (1899).

³ Lee, K. Y., R. Wahl, and E. Barbu, *Ann. Inst. Pasteur*, **91**, 212 (1956). Base compositions are those given by Lee *et al.* except for *E. coli* which was not reported by them. Data for *E. coli* is from Smith, J. D., and G. R. Wyatt, *Biochem. J.*, **49**, 144 (1951).

⁴ The mean buoyant densities of calf thymus, salmon sperm, and human leukocyte DNA all lie close to the values calculated from equation (1). However, the density distribution of the DNA from each of these vertebrate sources exhibits marked skewness toward higher density (M. Meselson, Thesis, California Institute of Technology, 1957). This skewness might reflect an uneven distribution of GC among the molecules of DNA or may be due to an uneven distribution of the rare base 5-methyl cytosine which occurs in small amounts in the DNA from these three sources.

The density composition relationship (1) is not valid for every DNA which has been examined. Density values considerably higher than those calculated from (1) are observed for heat denatured DNA from *E. coli*, calf thymus, and salmon sperm,¹ for T2 and T4 phage DNA in which cytosine is replaced by 5-hydroxymethyl cytosine or glucosylated 5-hydroxymethyl cytosine, and for DNA from the phage ϕX 174, found to have an unusual structure and composition [Sinsheimer, R. L., *J. Molec. Biol.* (in press)]. These exceptions indicate that the density composition relationship is valid only for a particular class of DNA, possibly for native two-stranded DNA containing no unusual bases.

⁵ It might be of interest to compare our estimate of the upper bound on the standard deviation σ_{GC} with the value to be expected for a population of DNA molecules each containing N base pairs assembled at random from a collection of base pairs in which guanine-cytosine occurs with fixed probability one half.

The standard deviation σ_{GC} for this case is just $\frac{1}{2\sqrt{N}}$. Taking 10⁴ as a reasonable value of N

for bacterial DNA¹ there results the value $\sigma_{GG} = 5 \times 10^{-3}$, well below the upper limit set by the density gradient experiments. Accordingly, such statistical heterogeneity is not ruled out.

*STRAIN SPECIFICITY AND PRODUCTION OF ANTIBIOTIC
SUBSTANCES. X. CHARACTERIZATION AND CLASSIFICATION OF
SPECIES WITHIN THE STREPTOMYCES GRISEUS GROUP*

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In 1948, a detailed description of the streptomycin-producing organism was published¹³ under the name *Streptomyces griseus* (Krainsky) Waksman and Henrici. In the eleven years that have since passed, there has accumulated a very extensive literature dealing with this organism and a number of related forms. Numerous varieties have been described, and a certain degree of confusion has arisen. It was thereby established that we are dealing here not with a single species, but with a group of closely related forms. Because of the great economic importance of this group, its re-evaluation and reinterpretation is justified at this time.

Nomenclature of the Streptomycin-Producing Organism.—In 1916, Waksman and Curtis¹¹ isolated from different soils cultures of an organism which they believed to be similar to a culture previously isolated and described as *Actinomyces griseus* by Krainsky.⁵ Waksman¹⁰ soon recognized, however, that the two organisms were slightly different. Since no type culture of Krainsky's organism has ever been available for comparison, all the descriptions subsequently published in the various editions of Bergey's *Manual of Determinative Bacteriology* were based upon that of Waksman and Curtis,¹¹ later modified by Waksman,¹⁰ and by Waksman *et al.*¹³ When Waksman and Henrici¹² proposed, in 1943, to create a new genus *Streptomyces*, this organism was named *Streptomyces griseus*. The strain to which this name was applied carries our laboratory number 3463. It was the first streptomycin-producing culture and has been designated as the type culture (by original designation) of the new species *Streptomyces griseus* Waksman and Henrici.

The original proposal to create a new generic name *Streptomyces* was made by Waksman and Henrici in a manuscript submitted to the *Journal of Bacteriology* in April, 1943, and to Dr. R. S. Breed in May, 1943, for publication in the sixth edition of Bergey's *Manual*. This name first appeared in the *Journal of Bacteriology* in October, 1943; unfortunately, the sixth edition of Bergey's *Manual*, which contained a detailed description of *Streptomyces griseus* was not published until 1948.

Krassilnikov⁶ also recognized a difference between the culture isolated by Krainsky and that of Waksman and Curtis. The organisms exhibiting spiral formation were regarded as characteristic of Krainsky's culture; the nonspiral forming strains characteristic of the streptomycin-producing *S. griseus* were regarded as a subspecies of *A. globisporus*, and designated as *A. globisporus streptomycini*, later changed to *A. streptomycini*.⁷ Krainsky's culture, however, has never been seen by