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A Procedure for the Isolation of Deoxyribonucleic Acid from Micro-organisms†

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A method has been described for the isolation of DNA from micro-organisms which yields stable, biologically active, highly polymerized preparations relatively free from protein and RNA. Alternative methods of cell disruption and DNA isolation have been described and compared. DNA capable of transforming homologous strains has been used to test various steps in the procedure and preparations have been obtained possessing high specific activities. Representative samples have been characterized for their thermal stability and sedimentation behaviour.

1. Introduction

To facilitate the study of the biological, chemical and physical properties of DNA it is necessary to obtain the material in a native, highly polymerized state. Several procedures have described the isolation of DNA from selected groups of micro-organisms (Hotchkiss, 1957; Zamenhof, Reiner, DeGiovanni & Rich, 1956; Chargaff, 1955). However, no detailed account is available for the isolation of DNA from a diverse group of micro-organisms. The reason for this is that micro-organisms vary greatly in the ease with which their cell walls can be disrupted, in their content of capsular polysaccharides (which are difficult to separate from DNA) and in the association of DNA to protein which influences the ease of DNA purification (Kirby, 1957). Most of these difficulties have been overcome in the present procedure which has been applied successfully to approximately 50 different species of micro-organisms. Included in this number are those organisms whose DNA can transform homologous and closely related strains and that have thus provided a very useful tool in determining the efficacy of many of the steps outlined in the procedure.

In general, the method to be described can be outlined as follows: the cells are first disrupted, the cell debris and protein removed by denaturation and centrifugation, the RNA removed by RNase and the selective precipitation of the DNA with *iso*-propanol. Degradation by DNase and divalent metal ion contamination is prevented by the presence of chelating agents and by the action of sodium lauryl sulfate. The products obtained, although they may not reflect the *in vivo* molecular weight, have molecular weights in excess of 6×10^6 and a high specific transforming activity, where this is present.

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2. Materials and Methods

(a) *Reagents*

Saline-EDTA, 0·15 M-NaCl plus 0·1 M-ethylenediaminetetra-acetate (EDTA), pH 8. The EDTA, and/or high pH, inhibit DNase activity.

Sodium lauryl sulfate, 25%. The anionic detergent ($NaC_{12}H_{26}SO_4$) will lyse most non-metabolizing cells, inhibit enzyme action and denature some proteins (Bayliss, 1937; Bolle & Kellenberger, 1958).

Lysozyme, crystalline (Armour). Used to lyse cells resistant to detergent action. Cells lysed with lysozyme are then subjected to sodium lauryl sulfate as well.

Sodium perchlorate, 5 M. The high salt concentration provided by the perchlorate (Lerman & Tolmach, 1957) helps dissociate protein from nucleic acid.

Chloroform-isoamyl alcohol, 24 : 1 (v/v). Used to deproteinize, according to the method of Sevag, Lackmann & Smolens (1938). The chloroform causes surface denaturation of proteins. The *iso*amyl alcohol reduces foaming, aids the separation, and maintains the stability, of the layers of the centrifuged, deproteinized solution.

Ethyl alcohol, 95%. Used to precipitate nucleic acids following deproteinization. Denatured ethyl alcohol may also be used.

Saline-citrate, 0·15 M-NaCl plus 0·015 M-trisodium citrate, pH 7·0 ± 0·2. Maintains ionic strength of dissolved DNA and chelates divalent ions.

Dilute saline-citrate, 0·015 M-NaCl plus 0·0015 M-trisodium citrate. DNA dissolves more readily in dilute salt solutions but should *never* be dissolved in pure water.

Concentrated saline-citrate, 1·5 M-NaCl plus 0·15 M-trisodium citrate. The concentrated solution is used to bring the dilute saline-citrate solute, in which the nucleic acid is dissolved, up to saline-citrate concentration. The volume added need only be approximate until the final pure product is obtained.

Ribonuclease, 0·2% (crystalline, Armour) in 0·15 M-NaCl, pH 5·0. The solution is heated at 80°C for 10 min to inactivate any contaminating DNase. The RNase digests the RNA and facilitates its separation from DNA.

Acetate-EDTA, 3·0 M-sodium acetate plus 0·001 M-EDTA, pH 7·0. This provides the proper ionic environment in the *iso*propanol step for the separation of DNA from RNA or its digestion products (Simmons, personal communication).

Iso*propanol*. Used to precipitate DNA selectively; RNA remains in solution. In some cases it will selectively precipitate and separate DNA from polysaccharides.

(b) *Equipment for DNA isolation*

Centrifuges, Servall SS-1 operating at 5 to 10,000 rev/min (3,000 to 13,000 ***g***) and a clinical swinging bucket centrifuge capable of spinning at 2 to 3000 rev/min (300 to 600 ***g***).

Glass-stoppered flasks, for deproteinization.

Shaker, wrist action or reciprocal for deproteinization. Several hundred strokes/min.

Volumetric pipette, 10 to 15 ml. fitted with an 18 in. (approx.) rubber tube attached to the upper end. Used to remove the aqueous layer from the deproteinized, centrifuged mixture.

Stirring motor, fitted with a glass stirring rod with a screw taper. Used to stir the solution (500 to 1,000 rev/min) during the *iso*propanol addition.

(c) *Physical and biological measurements*

Determination of T_m. The method has been previously described (Doty, Boedtker, Fresco, Haselkorn & Litt, 1959; Marmur & Doty, 1959).

Determination of sedimentation coefficient and molecular weight. The sedimentation coefficient, $S_{20,w}$, of DNA dissolved in standard saline-citrate was determined in the Spinco ultracentrifuge model E at a concentration of 20 μg/ml. at a speed of 35,600 rev/min using ultraviolet optics. The centrifuge cell was fitted with a Kel-F centerpiece. The molecular weight of the sample can then be obtained using the relationship established by Doty, McGill & Rice (1958):

$$S_{20,w} = 0{\cdot}063\, M_w^{0.37}$$

Transformation. The transformation of *Diplococcus pneumoniae* was carried out by the the method of Fox & Hotchkiss (1957) using transformable, glycerol-treated, cells stored at −20°C. When it was found necessary to examine the biological properties of *Bacillus subtilis* DNA, the method of Spizizen (1959) was used to transform this organism.

(d) *Isolation procedure*

The procedure is designed for 2 to 3 g wet packed cells. The volumes are only approximate unless otherwise stated. All operations can be performed at room temperature except the RNase treatment, which is carried out at 37°C.

Bacteria grown to the logarithmic phase of their growth cycle are harvested by centrifugation and washed once with 50 ml. saline-EDTA. After collecting by centrifugation, the cells are suspended in a total volume of 25 ml. of saline-EDTA. Lysis [1,2,3],† is effected by the addition of 2·0 ml. sodium lauryl sulfate and the mixture placed in a 60°C[4] water bath for 10 min then cooled to room temperature. Lysis of the culture results in a dramatic increase in viscosity[5] accompanying the release of the nucleic acid components and some clearing. If, on preliminary testing the cells are insensitive to the detergent, but sensitive to lysozyme, approximately 10 mg of lysozyme are added to the cells suspended in saline-EDTA. The mixture is then incubated at 37°C with occasional shaking and the lysis followed by noting the increase in viscosity. In some cases 30 to 60 min may be required for optimum results. When lysozyme is used sodium lauryl sulfate is added as well, *after* the cells have lysed, followed by the 60°C heating and cooling.

Perchlorate is added to a final concentration of 1 M to the viscous, lysed suspension and the whole mixture shaken with an equal volume of chloroform-*iso*amyl alcohol in a ground-glass stoppered flask for 30 min.[6] The resulting emulsion[7] is separated into 3 layers by a 5-min centrifugation at 5,000 to 10,000 rev/min in the Servall. The upper aqueous phase contains the nucleic acids and is carefully pipetted off into a tube or narrow flask. The nucleic acids are precipitated by gently layering approximately 2 vol ethyl alcohol on the aqueous phase. When these layers are gently mixed with a stirring rod, the nucleic acids "spool" on the rod as a threadlike precipitate[8] and are easily removed. The precipitate is drained free of excess alcohol by pressing the spooled rod against the vessel. The precipitate is then transferred to approximately 10 to 15 ml. of dilute saline-citrate[9] and gently removed from the stirring rod by swirling it back and forth. The solution is gently shaken or pipetted until dispersion is complete (lumps can be recognized by adhering air bubbles when the solution is shaken). The solution is adjusted approximately to standard saline-citrate concentration by adding concentrated saline-citrate, shaken as before with an equal volume of chloroform-*iso*amyl alcohol for 15 min, centrifuged[10] and the supernatant removed. It is then deproteinized repeatedly with chloroform-*iso*amyl alcohol,[11] as described, until very little protein is seen at the interface.

The supernatant obtained after the last in the series of deproteinizations, is precipitated with ethyl alcohol and dispersed in saline-citrate (about 0·5 to 0·75 the supernatant volume) in the manner already described. Ribonuclease[12] is added to a final concentration of 50 μg/ml. and the mixture incubated for 30 min at 37°C. Following the digestion of the RNA it becomes possible to remove protein which resisted earlier chloroform deproteinizations. The digest is again subjected to a series of deproteinizations until there is little or no denatured protein visible at the interface

† Numbers refer to the following section on Procedure notes.

after centrifugation. The supernatant, after the last such treatment, is again precipitated with ethyl alcohol and the drained nucleic acid dissolved in 9·0 ml. dilute saline-citrate. When solution has occurred, 1·0 ml. acetate-EDTA is added and while the solution is rapidly stirred, 0·54 vol[13] *iso*propyl alcohol is added dropwise into the vortex. The DNA usually precipitates in a fibrous form after first going through a gel phase at about 0·5 vol *iso*propyl alcohol. RNA or oligoribonucleotides and cellular or capsular polysaccharides remain behind, while the DNA threads wind around the glass propeller. If the yield is good, the DNA is redissolved and precipitated once more with *iso*propanol in the manner described. The final precipitate is washed free of acetate and salt by gently stirring the adhered precipitate in progressively increasing (70 to 95%) portions of ethyl alcohol, and is then dissolved in the solvent of choice. If the solution is not clear, it can be clarified by centrifuging in the Servall centrifuge for 10 min at 5,000 rev/min.[14]

By using caution and recovery steps, up to 50% of the DNA from the cell is obtained; in general 1 to 2 mg of DNA is obtained from 1 g wet packed cells.[15] The DNA can be stored in solution at 5°C in the presence of several drops of chloroform.[16,17,18] If it is so desired, the DNA (free of spores) can be sterilized by exposure to 75% ethyl alcohol for several hours and then transferred to a sterile solvent.

Notes on the procedure for the isolation of DNA

It would be very difficult to describe a definitive technique for the efficient isolation of DNA from a wide variety of micro-organisms. The method described can undoubtedly be modified or improved to eliminate difficulties encountered with specific strains. In general, the Gram negative organisms yield themselves readily to the procedure resulting in good recoveries of highly polymerized DNA. Several suggestions are offered to improve yields and eliminate some of the difficulties that may arise.

1. A spot test on a centrifuged portion of the culture should be made to determine whether the organisms are susceptible to sodium lauryl sulfate, lysozyme or neither of the two. If lysed by both detergent and enzyme, the former is preferable since DNase is inactivated in its presence. If lysozyme is used, the detergent is added *after* maximum enzymatic lysis is attained.

2. Organisms that are readily lysed by sodium lauryl sulfate include:— all Gram negative strains thus far encountered (Enterobacteriaceae, *Hemophilus influenzae*, *Rhizobium Japonicum*, *Pseudomonas aeruginosa*, *Pasteurella pestis*) as well as *D. pneumoniae* (which also lyses readily with deoxycholate) *Bacillus stearothermophilus*, *B. macerans*, *B. brevis*, *B. licheniformis*, *Clostridium madisoni*, *Cl. Chauvei*, *Cl. butylicum*, *Rhodospirillum rubrum*, *Micrococcus lysodeikticus* and *Mycoplasma* (PPLO). *Euglena gracilis* and *Chlamydomonas reinhardii* and the slime mold *Dictyostelium discoideum* are readily lysed by the detergent. Some strains of *Streptococcus* and of *M. pyogenes* var *aureus* lyse slowly with sodium lauryl sulfate and cell disruption is sometimes facilitated by raising the temperature of the lysing mixtures to between 70° and 75°C. The former genus can also be lysed by extracts from *Streptomyces albus* (McCarty, 1952).

The organisms lysed with lysozyme include: *B. subtilis*, *B. natto*, *B. cereus*, *B. megaterium*, *Cl. perfringens* as well as the Actinomycetes (*S. albus* and *S. viridochromogenes*) which lyse very slowly with the enzyme (Sohler, Romano & Nickerson, 1958), depending on the state of growth when harvested.

It has been found that some strains of *Streptococcus* and of *M. pyogenes* var *aureus*,

Lactobacillus acidophilus and baker's yeast are insensitive to lysis by either lysozyme or detergent. Yeast cells can be lysed by an extract from the snail *Helix pomatia* (Eddy & Williamson, 1957). If no means of enzyme or detergent lysis is available, the cells can be disrupted by grinding with alumina or glass powder (see below). The product, however, usually has a lower molecular weight than that obtained by the other methods described. (For other means of cell wall disruption see review by Weibull, 1958.)

The method has been successfully employed in the isolation of DNA from animal tissue, using sodium lauryl sulfate to lyse the cells.

3. It has been noted that some cells grown in the presence of 5-bromodeoxyuridine have altered their susceptibility to lysis. Thus, *D. pneumoniae* grown in the presence of this analogue will lyse slowly if at all with sodium lauryl sulfate, but remains susceptible to deoxycholate. This situation has not arisen in the case of *E. coli*.

4. If potent nuclease action is anticipated (e.g. *Serratia marcescens*), the detergent lysed suspension should be heated to within 10°C of the DNA T_m to eliminate its activity.

5. Some organisms (e.g. *Klebsiella pneumoniae*) are difficult to harvest and when lysed give rise to extremely viscous solutions. This is due to polysaccharide which is usually eliminated in the final stages of the DNA isolation but may also be removed earlier by initial use of the *iso*propanol step.

6. The shaking in the first deproteinization is carried out for 30 min because of the high viscosity of the lysate; subsequent steps are for 15 min.

7. A very critical step in the procedure is the concentration of cells being lysed. Too low a cell concentration will give rise to losses in subsequent alcohol precipitation whereas, if the cell suspension is too thick, the first chloroform deproteinization will result in a "lumpy" emulsion. The lumps consist of denatured protein with large amounts of occluded DNA and form a voluminous middle layer when the mixture is centrifuged. This difficulty is easily remedied in subsequent preparations by lysing a more dilute suspension of cells. In order to recover DNA from the denatured protein layer, because of excessive occlusion or when a high yield of DNA is desired under normal circumstances, the residue is shaken with a 10 ml. portion of dilute saline citrate for 15 min, centrifuged and the precipitate obtained from the ethyl alcohol addition combined with the remainder of the preparation.

8. If difficulty is encountered in collecting a majority of the nucleic acid threads following ethyl alcohol addition or if no threads appear because of DNA degradation, the solution is subjected to a short centrifugation, the sediment dissolved in a small volume of dilute saline-citrate and the alcohol precipitate added to the remaining portion of the preparation.

9. When dissolving the fibrous nucleic acid precipitate it is well to keep the concentration of DNA at a level of about 0·2 to 0·8 mg per ml. Too low a concentration results in degradation (Hershey & Burgi, 1960) and loss of biological activity during handling. Higher concentrations are highly viscous and difficult to handle and disperse. In the early stages of the preparation, the redissolved precipitate gives rise to turbid suspensions; as the purification proceeds, the nucleic acid takes on a glassy appearance when being dispersed and results in clear solutions.

10. If the emulsion separates readily into two phases after shaking, centrifugation in a swinging bucket clinical centrifuge at 2 to 3000 rev/min is sufficient to clear the aqueous layer of chloroform and most of the denatured protein.

11. The best time to interrupt the procedure, if it should be necessary, is after any of the deproteinization steps. In this case store the uncentrifuged emulsion until the procedure can be resumed. The average length of time required to isolate purified DNA starting from the lysis of the cells is approximately 5 to 8 hr.

12. It is also possible to isolate RNA by omitting the RNase treatment, recovering the ethyl alcohol precipitates by centrifugation and saving the solution after the DNA is removed by the *iso*propanol step. It would however be best to carry out the *iso*-propanol step earlier in the procedure.

13. Several cases have been encountered where the *iso*propanol step does not precipitate the DNA when 0·54 vol have been added (*C. reinhardii* and in one case of DNA isolated from tobacco leaves). When this occurs, larger volumes of *iso*propanol should be added. At times the precipitate is granular and can be collected by centrifugation.

14. It has been repeatedly observed that the purified DNA isolated from some spore formers (e.g. *B. brevis*, *B. subtilis*, etc.) is contaminated with viable spores. The number of spores can be reduced or eliminated by harvesting the cells when spore formation is at a minimum, centrifuging the purified DNA for about 10 min at 10,000 rev/min in the Servall and/or treatment of the DNA with phenol (see 17).

15. The method can be applied to bacterial harvests of the order of 100 g but, to avoid the awkwardness of handling large volumes, the amount of reagents used can be scaled up less than proportionately.

16. If protein removal is incomplete, storage of the DNA solution in the presence of chloroform will sometimes leave a halo of denatured protein surrounding the interface of the solutions. The protein can be removed by centrifugation.

17. The introduction of the phenol method (Kirby, 1957) to isolate DNA prompted its use on purified DNA. Purified *D. pneumoniae* DNA shaken with water saturated phenol remained undegraded and did not show any apparent loss in biological activity. Trypsin and chymotrypsin likewise had little or no effect on the molecular weight or biological activity.

18. The amino acid content (other than glycine) of the purified DNA on acid hydrolysis is approximately 0·3 to 0·5% (determined by Dr. H. Van Vunakis on the Spackman, Stein & Moore (1958) automatic amino acid analyser). Typical ratios for absorption of the DNA at 260 : 230 : 280 mμ are 1·0 : 0·450 : 0·515.

(e) *Alternative methods of cell disruption and lyophilization*

Mechanical disruption

Various techniques have been described (Gunsalus, 1957) for the mechanical disruption of cells for the isolation of enzymes, nucleic acids, etc. An attempt was made to disrupt *D. pneumoniae* cells by grinding with glass powder as well as by sonic treatment and to isolate the DNA from the disrupted cells according to the method described above. Since these and other methods might be applied to cells that resist enzyme and detergent action, we have applied them to *D. pneumoniae* as a basis of comparison to the detergent method of cell lysis.

Grinding with glass or alumina (particle size 500 mesh) is carried out at 5°C. The harvested, washed cells (with saline EDTA) are placed in a pre-cooled mortar, an equal weight of glass powder (Fisher Scientific Co.) added and the mixture ground with a pestle for 5 to 10 min. Ten vols of cold saline-EDTA, containing 2% sodium lauryl sulfate, are added and the suspension centrifuged to remove glass and large cell

debris. The supernatant is then treated in the manner described above for the isolation of DNA.

Sonic treatment is also carried out in the cold at 5°C. Saline-EDTA washed cells are suspended in 10 vol saline-EDTA and placed in the cup of the sonic apparatus (Raytheon). The suspension is exposed to 9 kc (50 w) sound waves for 5 to 30 min. Sodium lauryl sulfate is then added, the suspension centrifuged to remove any cell debris and then subjected to the isolation procedure. If the DNA does not precipitate as threads upon ethyl alcohol addition, the nucleic acids are collected by centrifugation.

Lyophilization of cells

Lyophilization is a commonly used laboratory technique for the preservation of cells. It was thought of interest to examine the DNA isolated from *D. pneumoniae* which had been subjected to freeze drying. The method employed for the isolation of DNA from the lyophilized cells is the same as that described using sodium lauryl sulfate as the lysing agent.

(f) *Isolation of DNA by cesium chloride density gradient centrifugation*

The introduction of the cesium chloride density gradient technique by Meselson, Stahl & Vinograd (1957) has made it possible to separate RNA, DNA and protein under mild conditions. By selecting the proper density and conditions of centrifugation, the RNA collects at the bottom of the centrifuge tube, the protein floats on the top and the DNA bands at or near the center. Biologically active DNA can readily be located by assaying the transforming activity of various fractions. The technique can also be adapted to separate DNA samples differing in base composition (and thus in buoyant density).

As applied to the isolation of DNA from cell lysates, the following outline has been used. A concentrated cell suspension of *D. pneumoniae* is lysed by sodium lauryl sulfate and the mixture shaken with a concentrated solution of CsCl containing 0·005 M-tris buffer (2-amino-2-hydroxymethylpropane-1:3-diol) plus 0·005 M-EDTA, pH 8·0. After adjusting the density to 1·706 $\pm$ 0·002 g/ml., the mixture is centrifuged in lusteroid tubes in the model L Spinco preparative centrifuge at 30,000 rev/min in the SW-39 rotor at room temperature for 3 days. When the rotor head has coasted (unbraked) to a stop, the tubes are removed, secured firmly in a vertical position and a small hole bored in the bottom of the tube with a small-gauge needle. The collected fractions, when sufficiently diluted to eliminate the inhibitory effects of CsCl on transformation (1 : 200 final dilution in the case of *D. pneumoniae*), are then assayed for biological activity. The fractions showing peak activity are combined, dialysed and treated with RNase to purify further the DNA. In one experiment, the DNA thus isolated accounted for 80% of the ultraviolet absorbing material at 260 mμ.

3. Results

A representative group of DNA samples isolated from bacteria by either lysozyme or detergent cell rupture is listed in Table 1. The sedimentation coefficient (at 20 μg/ml.) and T_m values have been determined and recorded. (The sedimentation coefficient was the same regardless of whether the centrifuge cells—regular or synthetic boundary —were filled slowly with a syringe or a wide-tipped pipette.) Even though the organisms listed vary widely taxonomically as well as in the base composition of their DNA, the purified DNA samples have approximately the same sedimentation coefficients with

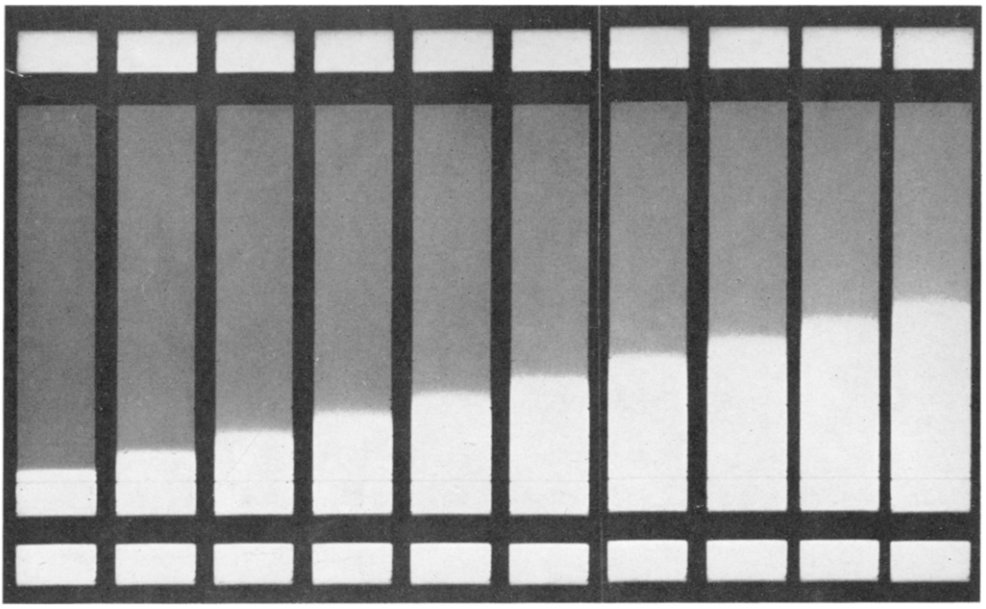

PLATE I. Ultracentrifuge sedimentation pattern of *E. freundii* DNA, 0·15 M-NaCl + 0·015 M-Na citrate. Using ultraviolet optics, pictures were taken every 4 min at 35,600 rev/min. First exposure at left. DNA concentration = 20 ug/ml.: 30 mm cell with Kel-F centerpiece. The sedimentation coefficient $S_{20,w} = 29{\cdot}4$.

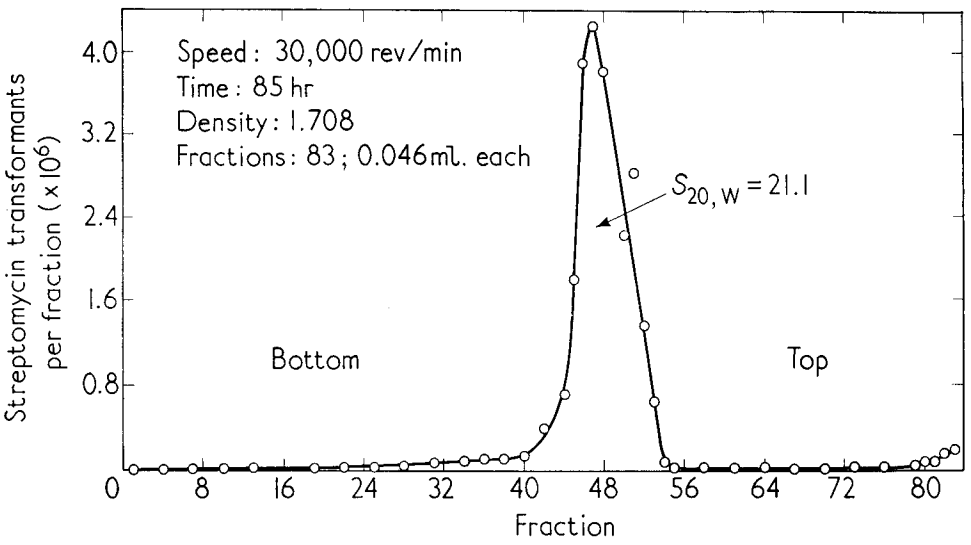

FIG. 1. CsCl density gradient isolation of DNA from streptomycin resistant *D. pneumoniae*. The ability to transform to streptomycin resistance is plotted as a function of the sample obtained by collecting drops from the punctured, lusteroid tube after centrifugation.

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calculated molecular weights of the order of 8 to 12 million. Calf thymus and salmon sperm DNAs, isolated and supplied by Dr. N. Simmons, are also listed for comparison. In determining the T_m values, the temperature-absorbance curves showed that all the DNA samples isolated were predominantly in the native configuration. Variations of the sedimentation coefficient and the T_m of different preparations of DNA from the same organism were of the order of $\pm$ 1 s and $\pm$ 0·3°C, respectively. An example of the sedimentation pattern of DNA isolated by the present method is shown in Plate I.

TABLE 1

Properties of representative DNA samples isolated from cells sensitive to lysozyme and/or sodium lauryl sulfate

Organism	$S_{20,w}$	T_m
Aerobacter aerogenes	22·4	94·0
Bacillus brevis	25·3	87·1
B. cereus	24·2	82·7
B. megaterium	29·0	85·2
B. subtilis	26·0	87·4
Diplococcus pneumoniae	24·0	85·2
Escherichia coli (K12)	29·0	90·0
Hemophilus influenzae	28·5	85·5
Klebsiella pneumoniae	25·6	92·0
Micrococcus lysodeikticus	24·0	98·6
M. pyogenes var *aureus*	28·0	83·2
Pseudomonas aeruginosa	28·2	96·7
Salmonella typhimurium	24·3	91·1
Serratia marcescens	23·7	93·6
Shigella dysenteriae	22·7	90·0
Streptococcus salivarius	24·4	85·2
Streptomyces albus	25·0	100·1
Calf thymus	22·1	86·0
Salmon sperm	23·2	87·0

The sedimentation coefficient and T_m were determined using saline-citrate (0·15 M-NaCl plus 0·015 M-Na citrate) as the solvent. The T_m for *S. albus* was obtained at a lower ionic strength and corrected to that for saline-citrate solvent. The sedimentation coefficients, determined immediately or within a day after the DNA preparation, did not alter on prolonged storage.

The effects of mechanical disruption and lyophilization of cells on the transforming activity and molecular weight of *D. pneumoniae* DNA are compared to detergent lysis in Table 2. Cells that have been subjected to freeze drying yield DNA with no detectable differences from that isolated from freshly harvested cells. However, as might be expected, sonic treatment (Litt, Marmur, Ephrussi-Taylor & Doty, 1958) has a deleterious effect on DNA. Grinding with glass is less destructive than sonic treatment and would thus be the preferred method of mechanical disruption. Other methods applying a shear force (Davison, 1959; Hershey & Burgi, 1960; Cavalieri & Rosenberg, 1959) would probably result in degradation of the DNA.

The result of the cesium chloride density gradient centrifugation technique for the isolation of DNA from *D. pneumoniae* is shown in Fig. 1. The fractions, assayed for

transformation with respect to streptomycin resistance, exhibit a peak activity at a density of CsCl expected from the base composition of the DNA (Sueoka, Marmur & Doty, 1959). The pooled active fractions, freed of contaminating RNA as described previously, had a high specific transforming activity and a molecular weight of 7×10^6 (estimated from the sedimentation coefficient). It is possible that some shear degradation of the DNA took place during mixing the lysate with CsCl, fraction collection through the narrow orifice or syringing the purified DNA into the sedimentation velocity cell (Davison, 1959). Similar experiments carried out by Mr. C. Schildkraut and Mr. R. Rownd (unpublished) employing milder conditions have yielded DNA from

TABLE 2

Effect of method of cell disruption and lyophilization on the transforming activity and molecular weight of D. pneumoniae *DNA*

Method of cell disruption	Relative transforming activity	Molecular weight $\times 10^{-6}$†
SLS‡	100%§	9·6
Grind with glass	36	5·0
Sonic 5′	4·1	1·0
Sonic 30′	0·42	0·38
Lyophilize, SLS	108	10·5

† Estimated from the sedimentation coefficient.

‡ SLS = sodium lauryl sulfate.

§ 100% represents $3{\cdot}6 \times 10^6$ transformants to streptomycin resistance per μg transforming factor.

E. coli with slightly higher sedimentation coefficients, in fact very similar to that for DNA isolated by deproteinization and alcohol precipitation as described. If links do exist between DNA molecules, they are disrupted by even the mildest conditions. Since the buoyant densities of the DNA in CsCl isolated by both methods are identical, one can conclude that both methods yield a product which contains the same amount of protein.

Several DNA samples were used to see whether they would transform homologous strains with respect to the streptomycin marker. Preliminary studies have shown that *E. coli* (K12), *E. freundii*, *M. pyogenes* var *aureus*, *Alcaligenes fecalis* and *Ps. aeruginosa* are incapable (less than 1 in 10^7) of being transformed, using techniques similar to those used for *D. pneumoniae* and *B. subtilis*.

4. Discussion

Micro-organisms possess DNAs that vary widely in base composition and thus offer an ideal source of this nucleic acid in the study of differences imposed on the molecule when the base pairs adenine : thymine and guanine : cytosine are present in different proportions. By studying the differences in the properties of DNA from different species, it is possible to gain an insight into the heterogeneity of the molecules comprising the genome of an organism.

The method described for the isolation of DNA has anticipated most of the difficulties that may be encountered with various micro-organisms. In general, the method described has yielded DNA from a variety of micro-organisms which is native, highly polymerized and possesses a fairly uniform molecular weight distribution. It might be argued that the method used for the isolation of DNA resulted in a degradation of the molecules and thus does not represent the true *in vivo* value. Degradation could have taken place during the shaking (Hershey & Burgi, 1960) used for deproteinization. The initial lysate is much more viscous than the final product; if the viscosity is due to a continuous DNA structure without protein links, then degradation has most likely taken place. The genetic evidence does indicate that the genome of *E. coli* behaves as one linkage group (Jacob & Wollman, 1958); however definitive evidence is still lacking as to whether this "chromosome" consists of large subunits or molecules (Forro & Wertheimer, 1960). Kellenberger (1960) has described a model in which protein "linkers" hold the DNA molecules together in the bacterial nucleoid.

The present method yields DNA from micro-organisms with a molecular weight of about 8 to 12×10^6 which is perfectly adequate for most experimental purposes. Those DNAs that possess transforming activity (e.g. *D. pneumoniae*, *B. subtilis*, etc.) can transform their homologous strains with an efficiency of 1 to 5% at saturating levels of DNA.

DNA isolated by selective buoyancy in a CsCl density gradient has physical, chemical and biological properties similar to the product obtained by the method described which involves selective denaturation with chloroform and alcohol precipitations. However, the DNA is still subjected to mild shear forces during the CsCl method and it is again possible that degradation may have taken place.

The methods of DNA isolation described in the literature, and here, are continually being improved and undoubtedly agents will be uncovered that will selectively precipitate or fractionate DNA under milder conditions than heretofore employed. The method presented in this report has been useful in providing samples of DNA from micro-organisms with widely varying base ratios and has proved of great value in studying their physico-chemical and genetic properties.

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