JOURNAL OF BACTERIOLOGY, Dec. 1972, p. 1033-1039
Vol. 112, No. 3

Purification of Mycobacterial Deoxyribonucleic Acid[1]

ELIZABETH B. HILL,[2] LAWRENCE G. WAYNE, AND WENDY M. GROSS

Division of Infectious and Tropical Diseases, School of Public Health, University of California, Los Angeles, California 90024, and Tuberculosis Research and Mycobacterial Reference Laboratories, Veterans Administration Hospital, Long Beach, California 90801

Received for publication 17 July 1972

Impurities believed to be polysaccharides have been found in mycobacterial deoxyribonucleic acid (DNA) preparations. Agar-gel diffusion of the DNA preparations against concanavalin A indicated the presence of three polysaccharides and was used to follow the purification procedures. The polysaccharides appeared to be the same for all strains studied. Precipitation of DNA with cetyltrimethylammonium bromide was used to separate impurities from some DNA preparations. The presence of the contaminants was found to affect markedly the determination of the guanine plus cytosine content according to a method dependent on the ratio of absorbancies at 260 and 280 nm; the impurities did not affect the determination by the method of thermal denaturation. The presence of a DNA-polysaccharide complex is suggested.

Impurities in mycobacterial deoxyribonucleic acid (DNA) preparations may interfere with studies of base composition or molecular hybridization of these products. In at least one instance, mycobacterial DNA was found to coprecipitate with an interfering substance in such quantities that the guanine plus cytosine (GC) content of the DNA could not be determined (16). The DNA preparations have an opalescent appearance rather than the clarity characteristic of pure DNA solutions. The absorption spectrum also indicates the presence of an impurity. Typical absorbancy ratios for solutions of DNA at 255 nm/280 nm and at 255 nm/230 nm are 2.0 and 2.2, respectively (10). Values for mycobacterial DNA are low, particularly for the 255 nm/230 nm ratio. The discrepancies between mycobacterial DNA absorbancy ratios and those ratios typical of other DNA preparations are not due to the presence of residual ribonucleic acid (15). Reaction with concanavalin A suggests that the interfering substance is probably polysaccharide.

This investigation is concerned with the separation of DNA from the co-precipitating substance. Concanavalin A in agar-gel diffusion was used to establish the presence of polysaccharides and to follow the progress of the purification procedures. Concanavalin A precipitates polysaccharides which have specific structural features (13). An arabinogalactan from *Mycobacterium bovis* cell walls has been reported to react with concanavalin A (7).

Precipitation of DNA with cetyltrimethylammonium bromide (CTAB) was used for the separation of mycobacterial DNA and polysaccharide. The relative amount of DNA and polysaccharide in the preparations was determined from poly-L-lysine kieselguhr (PLK) chromatography. Our studies suggest the existence of a DNA-polysaccharide complex. The relative effect of the presence of polysaccharide on the determination of the GC content by two different methods was studied.

MATERIALS AND METHODS

Organisms and isolation of DNA. Cultures from the collection of this laboratory were used: *M. kansasii* strains 1118, P1, and 427; *M. gastri* strain 417; *M. marinum* strain 467; and *M. tuberculosis* H37Rv strain 196. These organisms were grown and the DNA was isolated according to the method of Wayne and Gross (15). Highly polymerized *Bacillus subtilis* DNA was obtained from Nutritional Biochemicals Corp.

Precipitation of DNA with CTAB. The procedure of Darby et al. (5) was used, with some modifications. DNA was dissolved in 0.4 M NaCl, and 2 ml

[1] From a thesis submitted to the School of Public Health, University of California, Los Angeles, by the senior author, under Public Health Service training grant 05-T1-AI-00132-10, in partial fulfillment of the requirements for the M.S. degree.

[2] Present address: 1844 Forest Hills Blvd., Apt. 3, East Cleveland, Ohio 44112.

of CTAB, at a concentration of 5% in 0.4 M NaCl, was added per 5 ml of DNA. After 15 min, a small amount of CTAB was added to determine that precipitation of DNA was complete. The mixture was centrifuged, if necessary. The supernatant fraction was removed and treated with chloroform-isoamyl alcohol several times to remove CTAB, and was then dialyzed. The DNA-CTAB precipitate was washed two times with 0.4 M NaCl and dissolved in 1 M NaCl to recover the sodium salts of the nucleic acid. The solution was treated with chloroform-isoamyl alcohol several times until there was no precipitate at the interface. The DNA was then precipitated by adding three volumes of 95% ethanol. The DNA was dissolved in 0.7 M NaCl. CTAB, at a concentration of 5% in 0.7 M NaCl, was added to a final concentration of 1%. After 30 min, the mixture was centrifuged, and the supernatant fraction (DNA solution) was removed. The DNA was precipitated by adding three volumes of 95% ethanol. It was then dissolved in 0.1× standard saline citrate (SSC; 0.15 M NaCl plus 0.015 M sodium citrate) and treated with chloroform-isoamyl alcohol as above. The solutions were dialyzed against either distilled water or 0.1× SSC.

For those trials for which precipitation in 0.4 M NaCl was used without subsequent 0.7 M NaCl precipitation, the DNA product was dissolved in distilled water or 0.1× SSC and dialyzed against the solvent.

PLK chromatography. The procedure of Ayad and Blamire (1) was used to construct the three-layer column. A standard column of 1.1 by 25.7 cm was used. Each layer was suspended in 0.4 M NaCl-0.02 M KH_2PO_4 adjusted to *p*H 6.7 with 0.4 M NaCl-0.02 M K_2HPO_4. The first layer was 1.2 g of cellulose powder, the second layer was 6.0 g of kieselguhr plus 6.5 mg of poly-L-lysine, and the third layer was 0.12 g of kieselguhr alone. All layers were packed under air pressure of 2 psi. DNA was also loaded on the column under air pressure of 2 psi. A 10-ml amount of DNA was loaded at a concentration of approximately 100 μg/ml in 0.4 M NaCl-0.02 M KH_2PO_4 at *p*H 6.7, as calculated from the optical density at 255 nm. Approximately 400 μg/ml was used for one trial with *M. gastri* DNA. The 0.4 M NaCl-0.02 M KH_2PO_4 buffer was added to wash the sample. A linear gradient was used for elution of the DNA: 150 ml of 0.4 M NaCl-0.02 M KH_2PO_4 buffer and 150 ml of 4.0 M NaCl-0.02 M KH_2PO_4 buffer. The effluent was collected in 4-ml samples. The flow rate was approximately 25 ml/hr. The column was regenerated by washing with 0.4 M NaCl buffer until the effluent returned to 0.4 M chloride concentration, as determined by the method of Schales and Schales described by Henry (9).

Agar-gel diffusion. Agar-gel diffusion medium was composed of 0.85% NaCl, 0.00067 M phosphate buffer (*p*H 7.3), 0.5% sodium azide, and 1% Ionagar No. 2. The final *p*H was 7.6. For each diffusion plate with a diameter of 5 cm, 5 ml of diffusion medium was used. Wells (0.4 cm diameter, 0.8 cm between well centers) were cut in the agar plates in a circular pattern with six outer wells equally spaced from a center well.

Determination of GC values. GC values were determined by two different methods. For determinations based on thermal denaturation profiles, the DNA was dissolved in 10^{-2} M sodium phosphate-10^{-3} M ethylenediaminetetraacetic acid buffer at *p*H 7.0 (3); a concentration of approximately 20 μg/ml, as estimated by the absorbancy at 255 nm, was used. Thermal denaturation studies were completed according to methods used previously (16). The percent GC was calculated according to the relationship: $T_m = 51 + 0.45$ GC (3).

The method of Fredericq, Oth, and Fontaine (6) was also used to determine the GC content of the DNA. The DNA was dissolved in 0.1 N acetic acid, to a concentration of approximately 10 to 15 μg/ml. CTAB supernatant fractions, containing polysaccharide were dialyzed against and diluted in 0.1 N acetic acid to a concentration such that they gave the same absorbancy at 260 nm as a 10 to 15 μg/ml sample of DNA. The absorbancy at 260 and 280 nm was determined for each sample. Multiple readings were made for each sample, and the molar percent GC was determined from the ratio of absorbancy at 260 nm to absorbancy at 280 nm according to the graph of Fredericq et al. (6).

RESULTS

All mycobacterial DNA preparations isolated formed precipitation bands when diffused against concanavalin A (Fig. 1). Three bands were apparent for all preparations except for *M. gastri* DNA; three bands have been observed previously for *M. gastri* preparations. The precipitation bands for all strains tested

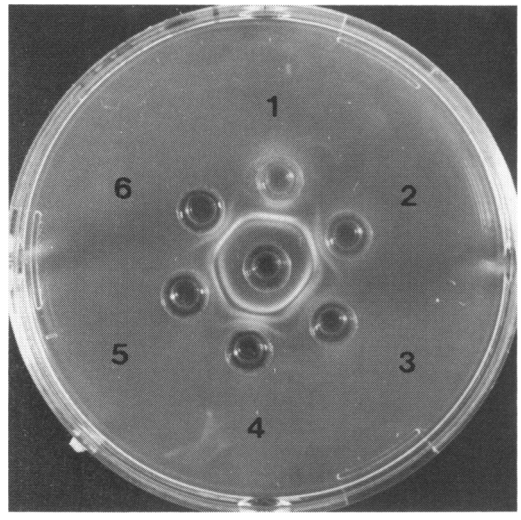

FIG. 1. *Initial isolation of DNA. Agar-gel diffusion pattern. Center well: concanavalin A, 5.0 mg/ml. Peripheral wells: (1) M. gastri DNA, (2) M. kansasii 427 DNA, (3) M. kansasii P1 DNA, (4) M. kansasii 1118 DNA, (5) M. marinum DNA, and (6) M. tuberculosis DNA.*

showed fusion with one another. The middle band appeared to represent the most concentrated polysaccharide, as it remained apparent after greater dilution than any of the others. A fourth precipitation band was observed occasionally in two *M. kansasii* preparations (1118 and P1); at some concentrations of DNA, it was obscured by the concentrated middle band. The commercially obtained *B. subtilis* DNA failed to produce precipitation lines when diffused against concanavalin A. The 260 nm/280 nm ratio of this DNA was 1.89.

CTAB precipitation of DNA. Precipitation of DNA with CTAB in 0.4 M NaCl was successful in removing contaminating polysaccharides from the DNA prepared from some strains of mycobacteria. The absorbancy ratios of the DNA preparations were increased to within acceptable ranges in most cases (Table 1). The increase was greater for the 255 nm/230 nm ratios. Experiments in which 0.7 M NaCl precipitation was used were not helpful in further improving the absorbancy ratios. Optical density analysis of the dialyzed CTAB supernatant fractions indicate that there was significant absorbance in the range of interest. The absorbancy ratios were consistently lower for the supernatant fractions than for the DNA preparations before CTAB precipitation.

TABLE 1. *CTAB precipitation at 0.4 M NaCl*

Sample	Absorbancy ratio	
	255 nm/ 230 nm	255 nm/ 280 nm
M. gastri DNA		
Before precipitation	0.94	1.59
After precipitation	1.38	1.64
M. gastri supernatant	0.77	1.47
M. kansasii 427 DNA		
Before precipitation	1.23	1.72
After precipitation	1.95	1.92
M. kansasii 427 supernatant	0.60	1.56
M. kansasii P1 DNA		
Before precipitation	0.96	1.61
After precipitation	1.95	1.83
M. kansasii P1 supernatant	0.68	1.50
M. kansasii 1118 DNA		
Before precipitation	1.32	1.67
After precipitation	1.99	1.92
M. kansasii 1118 supernatant	1.36	1.51
M. marinum DNA		
Before precipitation	1.37	1.64
After precipitation	1.83	1.86
M. marinum supernatant	0.58	1.40
M. tuberculosis DNA		
Before precipitation	1.51	1.75
After precipitation	1.82	1.88
M. tuberculosis supernatant	0.70	1.42

When agar-gel diffusion was used, two precipitation lines were observed for each of the recovered polysaccharide solutions (Fig. 2 and 3). The second line for the *M. tuberculosis*

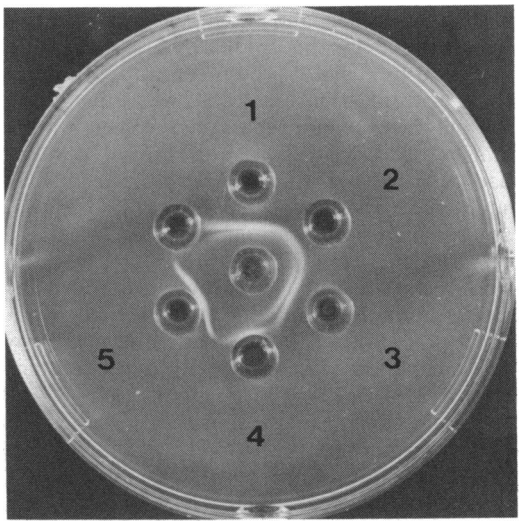

FIG. 2. *Dialyzed supernatant fraction from CTAB precipitation of DNA (concanavalin A at 5.0 mg/ml). Agar-gel diffusion pattern. Center well: concanavalin A, 5.0 mg/ml. Peripheral wells: dialyzed supernatant fractions from (1) M. gastri; (2) M. kansasii 427, (3) M. kansasii 1118, (4) M. marinum, and (5) M. tuberculosis.*

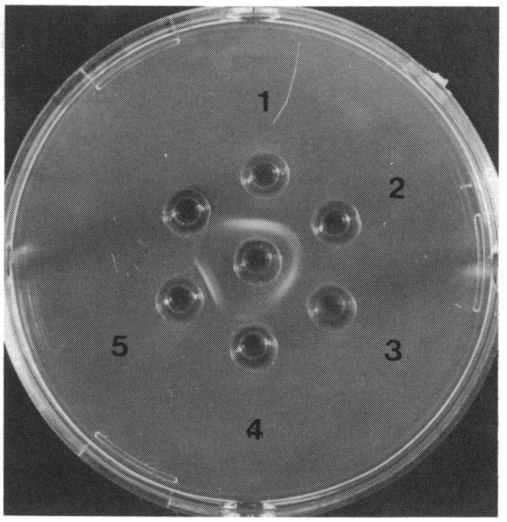

FIG. 3. *Dialyzed supernatant fraction from CTAB precipitation of DNA (concanavalin A at 2.5 mg/ml). Agar-gel diffusion pattern. Center well: concanavalin A, 2.5 mg/ml. Peripheral wells: dialyzed supernatant fractions from (1) M. gastri, (2) M. kansasii 427, (3) M. kansasii 1118, (4) M. marinum, and (5) M. tuberculosis.*

polysaccharide preparation was visible only with a more dilute concentration of concanavalin A. Only one precipitation line was visible for the *M. marinum* polysaccharide preparation. These precipitation lines suggest identity with each other and with the precipitation lines formed by the crude DNA preparations, as shown by fusion of the precipitation lines. A third precipitation line was not apparent in the polysaccharide preparations. When a preparation of *M. kansasii* strain 1118 DNA which had been treated with CTAB was concentrated such that the absorbancy at 255 nm was greater than that of the *M. kansasii* 1118 DNA preparation before CTAB precipitation, diffusion against concanavalin A at several concentrations did not indicate the presence of the third precipitation line. Presence of a trace of polysaccharide was suggested in the concentrated *M. kansasii* 1118 DNA after CTAB treatment by the deflection of a precipitation line from the *M. kansasii* 1118 DNA before CTAB precipitation (Fig. 4).

When *M. kansasii* 1118 DNA and *M. kansasii* 1118 polysaccharide which had been separated by CTAB precipitation were diffused against concanavalin A, a precipitation line formed between the DNA and the polysaccharide in adjacent outer wells (Fig. 4). The precipitate did not appear to be influenced by the concanavalin A, because there was no gradient along the precipitation line as the concanavalin A became less concentrated. When *M. kansasii* 1118 DNA was diffused against *M. kansasii* 1118 polysaccharide in the absence of concanavalin A, a precipitation line was observed. At a more dilute polysaccharide concentration, no precipitation line was apparent. At a 1:5 dilution of the DNA and the original concentration of polysaccharide, the precipitation line was still apparent, but at a 1:10 dilution of the DNA the precipication line was not observed.

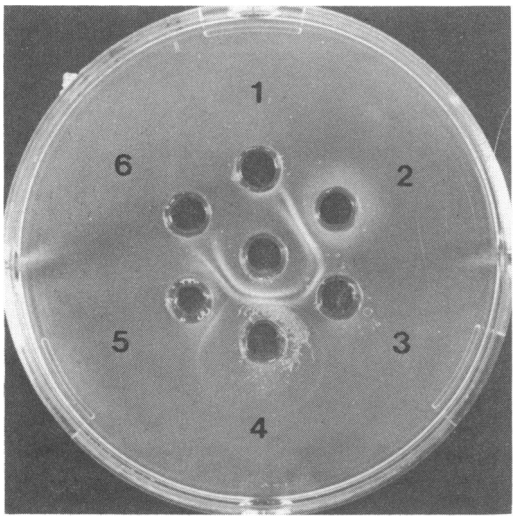

FIG. 4. *Products of CTAB precipitation. Agar-gel diffusion pattern. Center well: concanavalin A, 5.0 mg/ml. Peripheral wells: (1) M. gastri DNA after CTAB precipitation, (2) M. gastri DNA before CTAB precipitation, (3) M. gastri supernatant fraction, (4) M. kansasii 1118 supernatant fraction, (5) M. kansasii 1118 DNA before CTAB precipitation, and (6) M. kansasii 1118 DNA after CTAB precipitation.*

GC determinations. The DNA preparations and polysaccharides resulting from CTAB treatment were used in thermal denaturation studies. There were four preparations for which GC determinations were made both before and after CTAB precipitation. Values for percent GC determined from both types of preparations appeared to be very similar for a strain (Table 2). Although there was some difference between average values, values determined concurrently for *M. kansasii* 427 DNA before and after precipitation were almost identical (64.2 and 64.3 for before and after precipitation, respectively); in no samples were differences greater than 1% seen. The percentage rise was significantly higher for all DNA preparations after CTAB precipitation than before CTAB precipitation, as characteristic of a more highly purified sample.

A thermal absorbancy profile was determined for the *M. kansasii* 427 polysaccharide. A slight decrease in absorbancy (3%) was observed for the initial reading at 70 C relative to the absorbancy at room temperature. Subsequent increases in temperature caused no fur-

TABLE 2. *GC values of DNA as estimated by thermal denaturation*

Strain	Percent GC	Total percent rise in absorbancy
M. tuberculosis DNA		
Before precipitation	64.2	25.1
After precipitation	63.2	32.7
M. kansasii 1118 DNA		
Before precipitation	64.6	24.0
After precipitation	63.9	29.0
M. kansasii 427 DNA		
Before precipitation	63.6	22.8
After precipitation	64.2	32.0
M. marinum DNA		
Before precipitation	63.5	24.7
After precipitation	63.4	34.1

ther change in absorbancy of the dialyzed CTAB supernatant fraction. Thermal denaturation profiles of DNA preparations prior to CTAB treatment consistently showed a very slight decrease in absorbancy for the initial reading relative to the absorbancy at room temperature, occurring prior to the normal hyperchromic effect. This decrease had been overlooked previously, but it does not introduce error because it occurs before the DNA melt range. This decrease was not observed for those DNA preparations from which impurities had been removed by CTAB treatment.

The GC content of the DNA preparations was also estimated according to the ratio of absorbancies in 0.1 N acetic acid at 260 and 280 nm. Three sets of readings were made for each sample. The average values of GC content are presented in Table 3. The polysaccharides were treated as though they were DNA preparations for determining what would be the equivalent GC content. The polysaccharide preparations gave very low values for "percent GC." The purified DNA preparations gave higher values for percent GC in all cases than did the corresponding DNA preparations which still contained polysaccharides. These results indicate that the presence of impurities in the original DNA preparations acts to decrease the apparent value of percent GC determined by this method, by 2 to 3%.

PLK chromatography. The elution pattern from PLK columns depends on base composition, molecular weight, and secondary structure (8). *M. kansasii* 1118 DNA was first eluted from the PLK column by use of a stepwise chloride gradient. A peak having absorbancy ratios typical of mycobacterial polysaccharide separated by CTAB precipitation was eluted with the initial 0.4 M NaCl buffer. A second peak was eluted at a chloride concentration of 1.11 M. The effluent gave absorbancy ratios of 1.43 and 0.75 for 255 nm/280 nm and 255 nm/230 nm, respectively. A third peak was eluted at a chloride concentration of 1.68 M. Absorbancy ratios of 1.51 and 0.95 were seen at 255 nm/280 nm and 255 nm/230 nm, respectively. A fourth peak was eluted at a chloride concentration of approximately 2.0 M. Absorbancy ratios were 1.77 and 1.87 for 255 nm/280 nm and 255 nm/230 nm, respectively. Agar-gel diffusion against concanavalin A indicated that at least two of the first three peaks contained polysaccharide. Presence of a trace of polysaccharide in the *M. kansasii* 1118 DNA after elution was suggested by the deflection of a precipitation line from the crude DNA.

When *M. kansasii* 1118 DNA was eluted in a linear gradient, polysaccharide was eluted with the initial 0.4 M NaCl buffer (Fig. 5). A second peak was observed to elute at a chloride concentration of 1.40 to 1.50. The effluent gave absorbancy ratios of 1.90 and 1.88 for 255 nm/280 nm and 255 nm/230 nm, respectively, which are characteristic of DNA. The relative absorbancy attributable to DNA in the sample was calculated according to the relative area under the curve of the elution profile. By this method, 49.9% of the total absorbancy of the original *M. kansasii* 1118 preparation was shown to be contributed by the DNA peak. Similar work with *M. gastri* DNA indicated that only 20.1% of the absorbancy was contributed by the sample in the DNA peak. When the *M. gastri* DNA which was eluted from the PLK column was passed through the column a second time, the DNA contributed 33% of the total absorbancy.

TABLE 3. *GC values of DNA as estimated by absorbancy ratio in 0.1 N acetic acid*

Sample	Absorbancy ratio, 260 nm/280 nm	Molar % A	Molar % GC
M. kansasii 427 DNA			
Before precipitation	1.198	19.6	60.8
After precipitation	1.17	18.4	63.2
M. kansasii 427 supernatant	1.331	(25.0)[a]	(50.0)[a]
M. kansasii 1118 DNA			
Before precipitation	1.190	19.2	61.6
After precipitation	1.167	18.2	63.6
M kansasii 1118 supernatant	1.254	(22.0)[a]	(56.0)[a]
M. tuberculosis DNA			
Before precipitation	1.203	19.9	60.2
After precipitation	1.175	18.5	63.0
M. tuberculosis supernatant	1.312	(24.2)[a]	(51.6)[a]

[a] Dialyzed CTAB supernatant fraction behaves as if it had this base composition; it contributes to error by lowering the apparent percent GC of the DNA preparation.

DISCUSSION

All of our preparations of mycobacterial DNA appeared to be contaminated with some chemical moiety. The agar-gel diffusion studies support the belief that the contaminating material is polysaccharide. These studies indicate that there are at least three polysaccharides which precipitate with concanavalin A for all of the strains studies. The polysaccharides appear to be the same in all strains.

Concanavalin A has been reported to react with products derived from culture filtrates of several strains of mycobacteria (4, 7). Several polysaccharides have been isolated from myco-

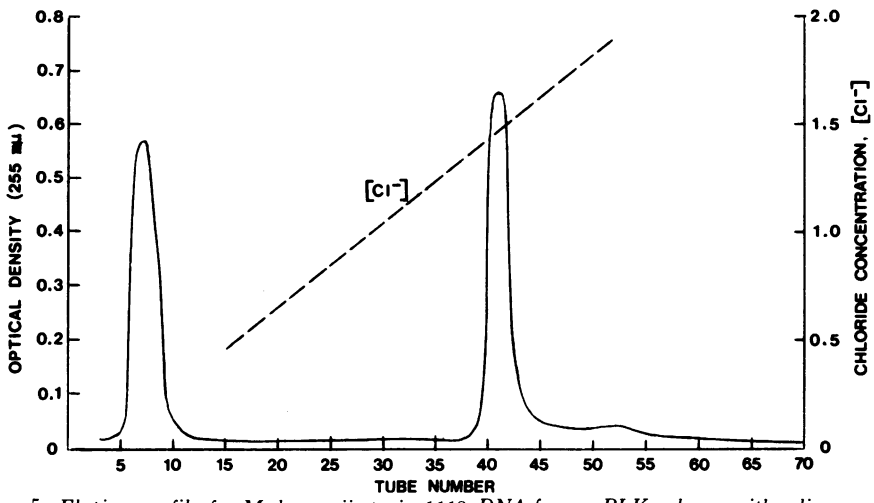

FIG. 5. *Elution profile for M. kansasii strain 1118 DNA from a PLK column with a linear gradient.*

bacteria which have the proper configuration for interaction with concanavalin A (2, 11, 12).

The CTAB precipitation studies indicate that this procedure may be used to separate DNA from contaminating polysaccharides from some strains of mycobacteria. Since the polysaccharides gave diffusion lines of identity for all DNA preparations studied, it is believed that they will be removed by CTAB at the same salt concentration for all strains. However, there may be a different proportion of each polysaccharide in different strains. Precipitation at 0.7 M NaCl yielded no evidence of the presence of acidic polysaccharide.

The absorbancy ratios of 255 nm/280 nm and 255 nm/230 nm were consistently lower for the dialyzed CTAB supernatant fractions than for the DNA preparations before CTAB precipitation. These results indicate that the low absorbancy ratios of the DNA are due at least in part to contamination by polysaccharide. The results of the PLK chromatography indicate the relative interference by polysaccharide in the DNA preparation. The *M. gastri* DNA, which is characterized by lower absorbancy ratios than the DNA of other mycobacteria, has a larger proportion of impurities than does the *M. kansasii* 1118 DNA. A minimum of 93% of the absorbancy in the DNA preparation isolated from the *M. gastri* bacilli was contributed by the impurities. In comparison, only 50.1% of the absorbancy of the original *M. kansasii* 1118 DNA preparations was contributed by these substances.

The results of GC determination for preparations before and after CTAB precipitation indicate that the presence of polysaccharides does not affect the percent GC calculated from thermal denaturation profiles. The average GC values for a strain before and after CTAB precipitation were within 1% GC of each other. The GC values for the DNA before CTAB precipitation were not consistently higher or lower than the values for the DNA after CTAB precipitation for the four strains studied. The "GC determination" of the dialyzed CTAB supernatant preparation indicates that the decrease in absorbancy due to polysaccharide occurs at a lower temperature than does the hyperchromic rise of native DNA preparations, so that it does not directly affect this method of determination of GC content.

Contamination with polysaccharide markedly interferes with the analysis of GC content by the method of Fredericq et al. (6). The values of molar percent GC for DNA preparations which are mixed with polysaccharide are 2 to 3% lower than those values for corresponding DNA preparations which have been purified. The dialyzed CTAB supernatant fractions give absorbancy ratios which correspond to a very low percent GC; this would contribute to the lowering of the value for the contaminated DNA.

The values of percent GC for the purified DNA preparations as determined by 260 nm/280 nm ratios are slightly lower than the values determined from the thermal denaturation profiles. The values for percent GC for the purified DNA preparations are higher than those determined by Tarnok, Rohrscheidt, and Bonicke (14) by the method of Fredericq et al. They reported 60.8% GC for *M. kansasii*. This corresponds to the values for the DNA from which the polysaccharide has not been separated. Their value of 65.2% GC for *M. tubercu-*

losis may be explained by a DNA preparation free from polysaccharides.

The differing elution profiles seen for the *M. kansasii* 1118 DNA with the stepwise and linear gradients in PLK chromatography suggest that the DNA and the polysaccharide are complexed in equilibrium with their separate components. This type of complex would allow the dissociation of polysaccharide at certain salt concentrations to establish a new equilibrium condition. The agar-gel diffusion pattern between the DNA and the polysaccharide also suggests that there is a complex between the DNA and the polysaccharide which resembles an antigen-antibody interaction. There is a region of optimal concentrations of DNA and polysaccharide where precipitation occurs in agar-gel diffusion. With excessive DNA or polysaccharide, precipitation does not occur. When DNA preparations initially isolated from the bacilli are placed in agar gel diffusion wells, an opalescent color is seen in the agar surrounding the wells. This opalescence may be due to the dissassociation and subsequent reassociation of the DNA-polysaccharide complex in such proportions to allow for precipitation. Further studies are necessary to define the nature of this complex.

LITERATURE CITED

1. Ayad, S. R., and J. Blamire. 1968. Fractionation of *Bacillus subtilis* DNA by use of poly-L-lysine kieselguhr columns. Biochem. Biophys. Res. Commun. **30**:207–212.
2. Azuma, I., M. Ajisaka, and Y. Yamamura. 1970. Polysaccharides of *Mycobacterium bovis* Ushi 10, *Mycobacterium smegmatis*, *Mycobacterium phlei*, and atypical *Mycobacterium* P1. Infect. Immunity **2**:347–349.
3. Bohacek, J., M. Kocur, and T. Martinec. 1967. DNA base composition and taxonomy of some micrococci. J. Gen. Microbiol. **46**: 369–376.
4. Daniel, T. M., and J. J. Wisnieski. 1970. The reaction of concanavalin A with mycobacterial culture filtrates. Amer. Rev. Resp. Dis. **101**:762–764.
5. Darby, G. K., A. S. Jones, J. F. Kennedy, and R. T. Walker. 1970. Isolation and analysis of the nucleic acids and polysaccharides from *Clostridium welchii*. J. Bacteriol. **103**:159–165.
6. Fredericq, E., A. Oth, and F. Fontaine. 1961. The ultraviolet spectrum of deoxyribonucleic acids and their constituents. J. Mol. Biol. **3**:11–17.
7. Goldstein, I. J., and A. Misaki. 1970. Interaction of concanavalin A with an arabinogalactan from the cell wall of *Mycobacterium bovis*. J. Bacteriol. **103**:422–425.
8. Helleiner, C. W. 1969. Fractionation of DNA on columns of poly-L-lysine supported on kieselguhr. Can. J. Biochem. **47**:1199–1202.
9. Henry, R. J. 1964. Clinical chemistry; principles and technics. Harper and Row, New York.
10. Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. **3**:208–218.
11. Seibert, F. B. 1949. The isolation of three different proteins and two polysaccharides from tuberculin by alcohol fractionation. Their chemical and biological properties. Amer. Rev. Tuberc. **59**:86–101.
12. Seibert, F. B., M. Stacey, and P. W. Kent. 1949. An antigenic polysaccharide, "Polysaccharide II," isolated from tuberculin. Biochim. Biophys. Acta **3**:632–640.
13. So, L. L., and I. J. Goldstein. 1967. Protein-carbohydrate interaction. IX. Application of the quantitative hapten inhibition technique to polysaccharide-concanavalin A interaction. Some comments on the forces involved in concanavalin A-polysaccharide interaction. J. Immunol. **99**:158–163.
14. Tarnok, I., E. Rohrscheidt, and R. Bonicke. 1967. Basenzusammensetzung der Desoxyribonuckleinsaure (DNS) von Mykobakterien und verwandten Mikroorganismen. Rass. Patol. Apparato Resp. **17**:3–16.
15. Wayne, L. G., and W. M. Gross. 1968. Isolation of deoxyribonucleic acid from mycobacteria. J. Bacteriol. **95**: 1481–1482.
16. Wayne, L. G., and W. M. Gross. 1968. Base composition of deoxyribonucleic acid isolated from mycobacteria. J. Bacteriol. **96**:1915–1919.