STUDIES ON NUCLEOPROTEINS

VI. THE DEOXYRIBONUCLEOPROTEIN AND THE DEOXYRIBONUCLEIC ACID OF BOVINE TUBERCLE BACILLI (BCG)*

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The first detailed chemical studies of a microbial deoxyribonucleoprotein and its deoxyribonucleic acid moiety, namely, the nucleoprotein of avian tubercle bacilli[2,3], brought to light several facts that gained added interest as our knowledge of nucleic acids increased. Among these were, on the one hand, the unusual composition of the nucleic acid, making it one of the extreme examples of the "GC type"[4], and, on the other hand, the uncommon stability of the conjugated protein; a resistance to dissociation into the protein and nucleic acid moieties that is reminiscent of the behavior of pentose nucleoproteins, but is not usually encountered in deoxypentose nucleoproteins. The characteristic composition of the deoxyribonucleic acid of acid-fast organisms has been confirmed repeatedly[4–8]. The outstanding feature, high guanine and cytosine values and low adenine and thymine values, has also been observed in a few other microbial species[4,8,9].

The present paper offers a more detailed investigation of the deoxyribonucleoprotein and the deoxyribonucleic acid of an acid-fast organism, the Bacillus Calmette-Guérin (BCG), an avirulent variant of *Mycobacterium tuberculosis*, bovine type. Particular attention was directed to the isolation of what could be considered a genuine nucleoprotein, to the investigation of the nature of its protein component and to the study of the preparation, the composition and the nucleotide arrangement of the nucleic acid.

EXPERIMENTAL AND RESULTS

Starting material and analytical procedures

The BCG strain of the National Institute of Health, Tokyo, was grown in surface culture on SAUTON medium[10] for 5 to 6 weeks. The organisms were collected by filtration, washed thoroughly with distilled water and dried in the frozen state in a vacuum.

Phosphorus was determined colorimetrically[11], protein by means of a modified biuret reaction[12] with crystalline pancreatic ribonuclease as the reference standard, polysaccharide through reaction with anthrone[13] or concd. sulfuric acid[14]. The methods used for the characterization of deoxyribonucleic acid have been reviewed[4]. Conditions of hydrolysis and analysis were, in general, those described in a previous paper[15]. A modification of the method of LEVY[16] was employed for the separation and estimation of the amino acids as the dinitrophenyl derivatives[17]

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Nucleic acid phosphorus distribution in BCG

One gram of the dry organisms, which formed our starting material, was found[18] to contain 0.4 mg of deoxyribonucleic acid phosphorus and 2.1 mg of ribonucleic acid phosphorus. This would correspond to an approximate content of 0.4 % of deoxyribonucleic acid and 2.3 % of ribonucleic acid. It is, however, well known[19] that in bacteria such distribution studies indicate the order of magnitude rather than the exact content.

Deoxyribonucleoprotein

Isolation. Most experiments were carried out in a cold room. The disintegration of the cells with Pyrex powder and their extraction with 0.1 *M* borate buffer (pH 8.4) followed the previously described procedure[2], except that in the present instance no preliminary ether extraction of the organisms was performed. As was done before, in each experiment 100 g of bacteria were extracted in four equal portions. The filtrate of the pooled supernatant fluids, collected by centrifugation, was adjusted to pH 4.2 with dilute acetic acid. The precipitate formed within three hours was twice more dissolved at pH 8.4 and brought back to pH 4.2. Its solution in a small quantity of borate buffer (pH 8.4) was subjected to dialysis against distilled water overnight and evaporated in a vacuum in the frozen state when the *crude nucleoprotein* (Fraction 1) was obtained. Analytical data for this and subsequent fractions are summarized in Table I. In the particular experiment listed in the table, Fraction 1 comprised 94 % of the bacterial deoxyribonucleic acid and 8% of the ribonucleic acid. The yields of this fraction ranged in different preparations from 3.5 to 4.4% of the dry weight of bacteria and the recovery of deoxyribonucleic acid from 75 to 94% of the amount contained in the organisms. The purification of the nucleoprotein will be described in the next section.

TABLE I

DEOXYRIBONUCLEOPROTEIN FRACTIONS AND DEOXYRIBONUCLEIC ACID OF BCG*

Fraction No.	*Yield*	*Total P*	*Extinction coefficient at absorption maximum*	*Nucleic acid distribution***		*Protein*
				DNA	*PNA*	
	% of dry organisms	*% of fraction*	*ε (P)*	*% of fraction*	*% of fraction*	*% of fraction*
1	4.4	1.3		8.7	4.3	
2	0.05	3.8	8200	31.5		
3	0.32	5.2	7900	46.5	6.5	
4	3.4	0.77		4.8	2.8	
5	0.25	7.7	7900	76.1	(7.6)	9.6
6	0.2	8.2	7000	94.5		3.3

* The values have been corrected for moisture content.

** Deoxypentose was determined by means of diphenylamine, pentose by means of orcinol, except in Fraction 5 in which the PNA content was calculated from the amount of P not accounted for as DNA.

The pooled supernatant fluids remaining after the precipitation of Fraction 1 at pH 4.2 were also examined. The precipitate produced by the addition of two volumes of ethanol was taken up in 1 *M* NaCl solution and deproteinized with Duponol[20]. The mixture was clarified by centrifugation and two volumes of ethanol

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were added to the supernatant liquid when a fibrous precipitate appeared which could easily be wound on a glass rod. The threads were once more treated with Duponol and precipitated with alcohol from their solution in 1 *M* NaCl, washed with alcohol and acetone, and dried in air. They are listed as Fraction 2 in Table I. This material represented a mixture of, or a complex between, deoxyribonucleic acid and polysaccharide. It was found to contain 39.6% of carbohydrate (estimated as glucose) when analyzed by means of anthrone[13] and 14.1% of glucose by the sulfuric acid method[14]. This fraction accounted for only about 4% of the bacterial nucleic acid. It was almost completely precipitated by a 2% lanthanum acetate solution; the sediment contained 92% of the nucleic acid and 82% of the polysaccharide components of Fraction 2.

Purification. The first step in the subsequent purification of the nucleoprotein made use of the previous observation that the conjugated protein is not precipitated at half-saturation with ammonium sulfate[2]. To a solution of 1.0 g of Fraction 1 in 1/15 *M* phosphate buffer of pH 7.3 the same volume of saturated $(NH_4)_2SO_4$ solution was added dropwise with stirring. The final pH was 6.0. Stirring was continued for 4 h and the mixture kept overnight. Centrifugation at 10,000 *g* for 20 min yielded a slightly turbid solution which was subjected to dialysis against running tap and distilled water for two days. The dialysis residue was concentrated in the flash evaporator to about one quarter of its volume and the remaining solution was, after dialysis against distilled water, evaporated *in vacuo* in the frozen state. The fibrous residue, designated Fraction 3 (Table I), weighed 70 mg*. It corresponds to the purified nucleoprotein fraction previously isolated from avian tubercle bacilli[2].

The material insoluble in ammonium sulfate solution was suspended with stirring in borate buffer (pH 8.4) and the solution was, after centrifugation and dialysis, lyophilized. The resulting white powder, listed as Fraction 4 (Table I), weighed 750 mg.

The deoxyribonucleic acid of the bacteria was about equally distributed in the two fractions, Fraction 3 accounting for 37.5 and Fraction 4 for 40% of the total.

Fractionation. Preparations corresponding in their stage of purity to Fraction 3 could be fractionated further by precipitation with ethanol at a low temperature. For this purpose, a 100 g batch of dry organisms was processed as described in the preceding section, but without the isolation of intermediate fractions in the dry state. After the step involving precipitation with $(NH_4)_2SO_4$ the solution containing Fraction 3 was recovered by centrifugation at 1500 *g* for 1 h (compare Footnote below) and concentrated in a vacuum to a volume of 70 ml. The solution was, after dialysis overnight against 500 ml of sodium acetate buffer of pH 5.8 containing sodium chloride (0.1 *M* with respect to both salts), placed in a thermoconstant bath kept at —10°. While the mixture was being stirred, ethanol was added dropwise till an ethanol concentration of 30% was reached. After 3 h in the cold bath, a small amount of precipitate was removed by centrifugation at 1500 *g* and —10° for 1 h. The opalescent supernatant solution was adjusted to an ethanol concentration of 50%, again at —10°, when a fibrous precipitate was formed. The mixture was kept overnight at —10° and the precipitate was collected by centrifugation at the same temperature, dissolved

* A better yield of Fraction 3 (13%, instead of 7%, of Fraction 1) can be obtained if the material insoluble at half-saturation with ammonium sulfate is removed by centrifugation at a lower speed (1500 *g*, 1 h), but a much more turbid supernatant fluid results.

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in ice-cold borate buffer of pH 8.4, subjected to dialysis, and recovered by lyophilization. It formed 300 mg of a white fiber felt. After removal of a sample for analysis the material was dissolved in a mixture of acetate buffer (pH 6.0) and NaCl (0.05 *M* with respect to both salts) and again subjected to fractional precipitation with ethanol at —10°. The fraction precipitable between a concentration of 35 and 50 % of ethanol was collected and recovered as described before. It weighed 246 mg and is listed in Table I as Fraction 5. The product of the first alcohol precipitation had almost identical analytical properties. Fraction 5 had an absorption maximum at 258 mμ with an ε (P) of 7900 and a minimum at 230 mμ with an ε (P) of 4000. When Fraction 5 was thus isolated directly, without the preparation of intermediates, the yield with respect to deoxyribonucleic acid varied in different specimens between 42 and 50 % of the nucleic acid content of the bacteria.

Amino acid composition. Specimens of Fraction 5, which contains 9.6 % of a protein component (Table I), served for the study of the amino acid composition of the nucleoprotein. After several preliminary experiments with two-dimensional chromatograms of hydrolysates and detection of the spots with ninhydrin, a quantitative estimation of the amino acids was carried out which made use of the chromatographic separation and spectrophotometric determination of the dinitrophenyl amino acids[16,17] liberated by hydrolysis with 6 *N* HCl (26 h at 110° in a sealed tube). Portions of 1.5 to 3 mg of Fraction 5 were used in each hydrolysis experiment. The relative proportions found are listed in Table II. An approximate calculation showed that about 70 % of the amino acids originally present had been recovered.

TABLE II

MOLAR PROPORTIONS OF IDENTIFIED AMINO ACIDS IN DEOXYRIBONUCLEOPROTEIN (Fraction 5) AND IN DEGRADATION PRODUCT AFTER PURINE REMOVAL*

Amino acid	*Nucleoprorein*	*Apurinic acid – protein degradation product*
Glutamic and aspartic acids	10	10
Glycine	8	4
Alanine	4	4
Histidine	4	3
Leucine and isoleucine	4	5
Arginine	3	< 1
Threonine	3	3
Valine	3	3
Proline	1	1
Serine	1	2
Lysine	< 1	< 1

* The sum of glutamic and aspartic acids is arbitrarily taken as 10.

The value for glycine required correction since this amino acid figures among the products of the acid degradation of purines[21]. For this purpose, a sample of Fraction 5 was treated under the conditions developed for the preparation of apurinic acid[22]. When 3.5 mg of Fraction 5 were thus treated with dilute HCl (pH 1.6) and simultaneous dialysis for 44 h at 37°, about 90 % of the adenine and 86 % of the guanine originally present were found in the dialysate. Dialysis was continued in distilled

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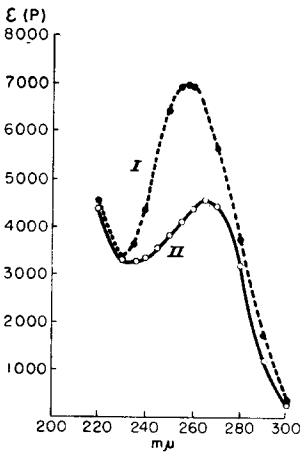
water and the dialysis residue was recovered by lyophilization. It contained P, 9.2 % and showed an absorption maximum at 265 mμ (ε (P) = 4950) and a minimum at 235 mμ (ε (P) = 3300). The spectrum is illustrated as Curve II in Fig. 1. The amino acid composition of this mixture of protein and apurinic acid was determined in the manner described before, with the results given in Table II.



It is possible to derive an approximate figure for the stoichiometric relationship between the amino acid and nucleotide residues in the deoxyribonucleoprotein. When the amino acid proportions found in Fraction 5 (Table II) are used, with the substitution of the relative glycine value of 4 (last column of Table II), an average amino acid-residue weight of 109 can be computed. The deoxyribonucleic acid phosphorus content of Fraction 5 is about 7.0 %. This would correspond to 0.4 amino acid residue for one nucleotide residue.

Fig. 1. Absorption spectra of the deoxyribonucleic acid of BCG, Fraction 6 (Curve I) and the apurinic acid–protein complex produced by the degradation of the nucleoprotein (Fraction 5) at pH 1.6 (Curve II); measurements in 0.1 *M* phosphate buffer of pH 7.2.

Deoxyribonucleic acid

Observations on protein removal. A previous study of the deoxyribonucleoprotein of avian tubercle bacilli[2] had pointed to a remarkable cohesiveness of the conjugated protein, as evidenced by the difficulty of separating the protein and nucleic acid moieties under conditions producing cleavage of nucleohistones and nucleoprotamines[4]. The same observation was made in the present instance. When the crude preparation, Fraction 1, was treated with sodium deoxycholate solution or chloroform-amyl alcohol in the arrangement of the previous investigation[2], the recovery of nucleic acid was unsatisfactory. The removal of protein from the purified nucleoprotein, Fraction 5, was studied with the use of three agents, namely, Duponol[20], phenol[23,24] and cryst. trypsin. The last agent was the most effective with respect to both the recovery and the purity of the nucleic acid.

Isolation. We describe one experiment. A mixture of 6 ml of a solution containing 57.1 mg of Fraction 5 and of 1 ml of a 0.1 % solution of crystalline trypsin (Worthington Biochemical Corp., Freehold, N.J.), both in 0.1 *M* borate buffer of pH 8.4, was placed in a cellophane bag and suspended, with mechanical agitation, in 10 vol. of the same buffer kept at 37°. After 6 h, during which time the outside fluid had been changed several times, the inside fluid was brought to a *M* NaCl concentration by the addition of solid salt, and two volumes of ethanol were added with stirring. The white fibers were collected, washed with 66 % ethanol and with acetone, and dried. They weighed 49.6 mg (87% of Fraction 5). A portion of this material, 28.4 mg, was dissolved in 3 ml of 0.1 *M* phosphate buffer (pH 6.5) and treated overnight at 30° with 0.3 mg of crystalline pancreatic ribonuclease, again with simultaneous agitation and dialysis against 20 vol. of the buffer. The dialysis residue was made 1 *M* with respect to NaCl and treated four times with chloroform–amyl alcohol (4:1). The solution was

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freed of dialyzable material, frozen, and evaporated in a vacuum. The isolated deoxyribonucleic acid preparation is listed as Fraction 6 in Table I. In this experiment the yield corresponded to 44% of the total deoxyribonucleic acid of the bacteria. The ε (P) of this preparation was 7000 at the absorption maximum, 258 mμ, and 3400 at the minimum, 230 mμ. The absorption spectrum is shown as Curve I in Fig. 1.

Composition. For analysis, samples of the deoxyribonucleic acid (Fraction 6) were hydrolyzed with 88 % formic acid at 175° in sealed tubes for 1 h. Previously described analytical methods[15] were used on four hydrolysates of two preparations. The results are summarized in Table III.

TABLE III

COMPOSITION OF DEOXYRIBONUCLEIC AND PENTOSE NUCLEIC ACIDS OF BCG

Proportions of nitrogenous constituents and molar ratios

	*DNA**	*PNA*
	Moles per 100 g-atoms P	*Moles per 100 moles nucleotide*
Adenine (A)	18.3	20.1
Guanine (G)	32.7	31.3
Cytosine (C)	32.3	29.4
Thymine (T)	16.7	
Uracil (U)		19.2
	Molar ratios	
Purines to pyrimidines	1.04	1.06
(A + T) to (G + C)	0.54	
(A + U) to (G + C)		0.65
(A + C) to (G + T)	1.02	
(A + C) to (G + U)		0.98

* The mean proportions of each constituent have been corrected for a 100% recovery. The actual total recovery of bases ranged from 90 to 95%.

Differential distribution analysis. The recently published method[25,26] was followed with only minor modifications, in order to characterize the distribution of pyrimidine nucleotides in the deoxyribonucleic acid by stepwise hydrolysis in 0.1 *M* H_2SO_4. A typical elution diagram is reproduced in Fig. 2. The eluted fractions were recovered and purified by chromatography on filter paper, as described before[26], and identified by base and phosphorus analysis after hydrolysis with 88 % formic acid. The fractions examined were the nucleoside 3',5'-diphosphates pCp and pTp and the dinucleoside triphosphates pCpCp and pCpTp + pTpCp (Table IV)*. The quantities of pCp and pTp released in the three stages of hydrolysis were estimated spectrophotometrically and, in Stages II and III, corrected for decay[26]. For the estimation of pCpCp an ε (P) value at 280 mμ of 2420 at pH 4, the pH of the eluates of this component, was used. The results of three experiments are averaged in Table V. The figures for pCpCp in Stages II and III were also corrected for the decomposition of this fragment occurring

* The presentation of structures follows a previous paper[25]. The deoxyribonucleosides are indicated by their initials: A, adenosine; G, guanosine; C, cytidine; T, thymidine. The position of the esterified phosphoric acid is indicated by p being placed at the right of the symbol if it is linked to the 5' hydroxyl of the nucleoside, at the left if it is on the 3' hydroxyl.

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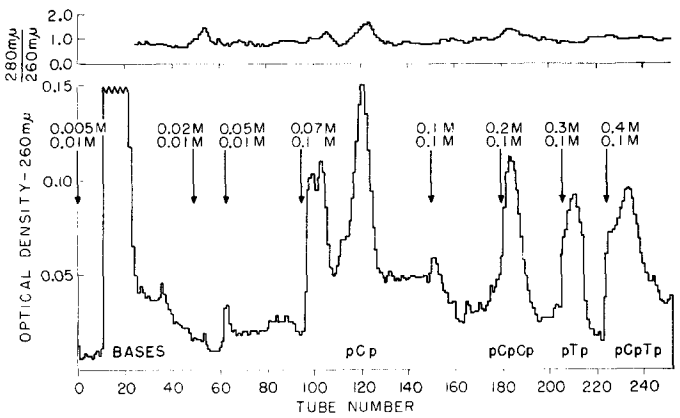

Fig. 2. Elution pattern of acid hydrolysate of BCG deoxyribonucleic acid. Conditions: 8 mg of Fraction 6, hydrolysis stage I (0.1 *M* H_2SO_4, 100°, 30 min). Dowex-2 formate (8 ×), 100–200 mesh; 10 cm × 0.5 cm diam.; 7.5 ml fractions. The concentrations of the elution mixtures are indicated, with the upper figure referring to ammonium formate, the lower figure to formic acid.

TABLE IV

FRACTIONS ISOLATED AFTER STEPWISE ACID HYDROLYSIS OF DEOXYRIBONUCLEIC ACID OF BCG

*Fraction**	*Molar ratio found*	
	Cytosine to phosphorus	*Thymine to phosphorus*
pCp	0.54	
pCpCp	0.62	
pTp		0.53
pCpTp + pTpCp	0.30	0.30

* See footnote for the presentation of structures, p. 573.

TABLE V

STEPWISE ACID HYDROLYSIS OF DEOXYRIBONUCLEIC ACID OF BCG

Intact DNA			
Moles thymine per 100 g-atoms P		16.7	
Moles cytosine per 100 g-atoms P		32.3	
Thymine to cytosine, molar ratio		0.52	
*Release of fragments**			
Stages	I	II	III
pTp, as mole % of total thymine	13.6	15.0	17.0
pTp, as mole % of total P	2.27	2.51	2.84
pCp, as mole % of total cytosine	12.0	13.2	14.4
pCp, as mole % of total P	3.88	4.26	4.65
pTp to pCp, molar ratio	0.59	0.59	0.61
pCpCp, as mole % of total cytosine	3.5	4.8	6.4
pCpCp, as mole % of total P	1.13	1.55	2.07

* The values for fragments liberated during the hydrolysis stages I, II, III (30, 60, 120 min, respectively, in 0.1 *M* H_2SO_4 at 100°) have been corrected for decay. The mole % figures refer to moles per 100 moles of total constituent in the intact DNA. See footnote on p. 573 for the presentation of structures.

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in the course of continued hydrolysis. The correction factor given before for the diphosphate pCp[26] was used for this purpose.

The components pCp and pTp appeared very rapidly within the first 30 min of hydrolysis and increased more slowly in the subsequent stages, with the hydrolysis rates of both pyrimidine nucleoside diphosphates remaining constant. The triphosphate pCpCp also accumulated in the hydrolysates. At the termination of the degradation, 27.2% of the total cytosine of the nucleic acid was found in the two fractions pCp and pCpCp.

As a supplement to the data assembled in Table V it may be of interest to note that the following figures for the liberation of inorganic phosphorus (as g-atoms per 100 g-atoms of total P in the nucleic acid) were recorded at the stated intervals of heating with 0.1 *M* H_2SO_4 at 100°: 30 min, 10.0; 60 min, 14.4; 90 min, 19.6; 120 min, 22.6.

Sugar moiety. The study of the deoxypentose component of the nucleic acid made use of previously established procedures[27,28]. To a solution of 2 mg of the deoxyribonucleic acid (Fraction 6) in 0.3 ml of Veronal buffer (pH 6.7), 0.1 ml of a solution of the enzyme mixture was added. The enzyme solution contained, per ml of the same buffer, 1.2 mg of crystalline pancreatic deoxyribonuclease (Worthington), 19 mg of intestinal phosphatase (Armour), and 185 μg of $MgSO_4 \cdot 7\ H_2O$. The assay mixture was incubated at 30° for 20 h, in the presence of some toluene, and then deproteinized by being shaken for 2 h with chloroform–amyl alcohol (4:1). The supernatant fluid, separated by centrifugation, was adjusted with *N* HCl to a pH of 1.6 and heated at 100° for 12 min. Portions of the hydrolysate containing about 10 μg of sugar were chromatographed in the four solvent systems listed before[29] and the adsorption zones were demonstrated by means of *m*-phenylenediamine[30]. In all chromatograms, the position of the sugar component of the BCG nucleic acid coincided with that of authentic deoxyribose released under identical conditions from calf-thymus deoxyribonucleic acid.

Pentose nucleic acid

For an orienting examination of the nucleotide composition of the pentose nucleic acid of BCG, 0.5-g portions of the dry organisms were ground for 15 min with Pyrex glass powder moistened with 7% aqueous trichloracetic acid. The residues were processed as described in a previous paper[31]. Average values for the nucleotide composition are included in Table III.

DISCUSSION

The deoxypentose nucleoproteins of microbial origin do not seem to accord with the pattern established for the nucleohistones and nucleoprotamines. This has been discussed on a previous occasion[4] when it was pointed out that there exist indications of the occurrence in nuclei of higher organisms of nucleoproteins carrying protein moieties of a more complex character than that of histone. The evidence has been surveyed in a recent paper[32]. The first example of such a nucleoprotein to be studied in some detail, in which the protein portion lacked basic properties and was firmly attached to the nucleic acid by bonds not broken by dissociation at a high salt concentration, is probably represented by the nucleoprotein of avian tubercle bacilli described previously[2]. In the present study of the corresponding conjugated protein

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from the Bacillus Calmette-Guérin (BCG), an organism of great therapeutic interest, a higher degree of purification could be attained. The first fractionation steps made use of some properties of the nucleoprotein established before[2], namely, its insolubility around pH 4 and its solubility at half-saturation with ammonium sulfate. Even more effective was the next step, the fractionation with ethanol at a low temperature; the nucleoprotein remained in solution at 30 % ethanol and was precipitated at 50 %.

A few of the properties of the deoxyribonucleoprotein are of interest. It contained what would appear to be an almost constant proportion of protein, around 10 %. As has been shown in a preceding section of this study, this corresponds to a stoichiometric ratio of 0.4 amino acid residue for one nucleotide residue. It is noteworthy that this is near the ratio that would be expected if one polypeptide chain were linked to two polynucleotide chains, so that each peptide link is bonded to one 6-amino group and to one 6-keto group of the two nucleotide chains. Such an arrangement has been discussed in detail previously in connection with the structure of ribonucleoproteins and with the one regularity common to both deoxypentose and pentose nucleic acids, namely, the equality of the molar concentrations of 6-amino nucleotides and of 6-keto nucleotides[33,34].

As concerns the amino acid composition of the nucleoprotein, the values listed in Table II should be considered as no better than approximations. They indicate that the dicarboxylic acids represent the largest component, outweighing the sum of the basic amino acids. The results reported here strengthen the former conclusion[2] that the protein associated with deoxyribonucleic acid in tubercle bacilli lacks basic properties. Moreover, most of the arginine found in the nucleotprotein is released by the very mild acid treatment that leads to the formation of apurinic acid (Table II); it may be present in a salt-like linkage.

In order to remove the protein component of the nucleoprotein, it was necessary to apply tryptic digestion. In this manner, highly polymerized deoxyribonucleic acid preparations could be obtained which were freed of residual pentose nucleic acid by treatment with ribonuclease. There exists, at any rate provisional, justification in referring to the deoxypentose nucleic acid of BCG as deoxyribonucleic acid: the sugar released by mild hydrolysis of its purine nucleoside constituents had chromatographic properties that were identical with those of deoxyribose in four solvent systems. The base composition of the deoxyribonucleic acid (Table III) permits its classification as being of an extreme "GC type"[4]; its dissymmetry ratio (A + T)/(G + C) of 0.54 brings it into line with the nucleic acids of other acid-fast microorganisms[4] and is similar to the ratio of 0.48 reported in another recent study of the same organism[8]. The normal compositional regularities of deoxypentose nucleic acids[4] were all present.

In the course of the present study we have attempted to indicate at most isolation stages the percentages of total bacterial deoxypentose nucleic acid recovered. This is of importance, and particularly in microorganisms that are refractory to the complete extraction of the nucleic acid, if a decision is to be reached on the degree to which the analytical findings can be considered as representative. There is, however, in general no evidence that the incomplete extraction of bacterial deoxypentose nucleic acids may lead to fractionation. For instance, deoxyribonucleic acid specimens of *E. coli* prepared under unfavorable conditions[4] gave nearly the same analytical values as did preparations from protoplasts that must have comprised the bulk of the bacterial nucleic acid[35].

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An attempt was also made, not described here in detail, to fractionate the deoxyribonucleic acid of BCG by the fractional dissociation of its salt with calf-thymus histone[36]. Three fractions were obtained, though in a total yield of only 47 % of the starting material, by dissociation at 0.7, 1.0 and 3.0 *M* NaCl concentration. These preparations did not, however, differ from each other analytically and exhibited nearly the same base distribution as the unfractionated nucleic acid. Scarcity of material prevented a more detailed investigation which, it is hoped, will be resumed on a later occasion.

In a previous study of details of the nucleotide arrangement in deoxypentose nucleic acids[26] attention was directed to the lack of information on nucleic acids of the "GC type". An investigation of some aspects of the distribution of pyrimidine nucleotides, as revealed by the stepwise hydrolysis of a specimen from BCG, was therefore undertaken (Fig. 2 and Tables IV and V). In the preceding study of the differential distribution analysis of nucleic acids of the "AT type" more thymidine-3',5'-diphosphate, pTp, was found to be released than deoxycytidine-3',5'-diphosphate, pCp, but in most instances not in yields or ratios that could have been predicted on the basis of the original pyrimidine contents of the specimens[26]. It was of interest to determine whether in a "GC type" with an inverted pyrimidine ratio, as is the case in the nucleic acid under study here, a corresponding inversion in the rates of liberation of the nucleoside diphosphates could be noticed. This is indeed the case; but the inspection of Table V will also show that the ratio of pTp/pCp is close to, but not identical with, the original T/C ratio, namely 0.59 in contrast to 0.52. The results, moreover, agree with the conclusions reached in the earlier investigation: the figures for the liberation of pyrimidine nucleoside-3',5'-diphosphates reflecting the number of "solitary" pyrimidine units, and for the release of the fragment pCpCp reflecting "coupled" cytidylic acid dimer units that are flanked by purine nucleotides (Stage I), are far below the figures suggested by the mathematical treatment of a random arrangement of nucleotides (pTp and pCp, 25 mole % of total thymine and cytosine, respectively; pCpCp, 6.25 mole % of total cytosine). The data for the dinucleoside triphosphate pCpCp supplement the information given for the other fragments in the previous papers[25,26].

The increments of pTp and pCp between Stages I and III point to a relatively slow liberation of these fragments from polypyrimidine units. The comparatively more rapid release of pCpCp between these stages indicates a significant breakdown of higher polypyrimidine fragments. A complete interpretation of the serial degradation of pyrimidine oligonucleotides is, however, not yet possible.

In conclusion, mention should be made of a preliminary study of the nucleotide composition of the pentose nucleic acid of BCG whose results are shown in Table III. The figures again demonstrate the equality of the molar concentrations of the 6-amino nucleotides (adenylic and cytidylic acids) and of the 6-keto nucleotides (guanylic and uridylic acids): a feature characteristic of most pentose nucleic acids which was discussed in a previous study[33].

SUMMARY

The preparation of a deoxyribonucleoprotein from the BCG variant of bovine type tubercle bacilli is described. The principal steps involved precipitation at pH 4.2, removal of contaminating materials at half-saturation with ammonium sulfate, and fractionation with ethanol at a low tem-

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perature. The purified product was shown to contain about 0.4 amino acid residue for one nucleotide residue. An approximate estimation of the principal amino acids contained in the nucleoprotein revealed a preponderance of dicarboxylic over basic amino acids.

The deoxyribonucleic acid was freed of its protein component by tryptic digestion and examined with respect to the distribution of purines and pyrimidines. It belongs to the extreme "GC type". More detailed aspects of the arrangement of nucleotides in the nucleic acid chain were studied through the application of the method of differential distribution analysis, making use of the results of the stepwise acid hydrolysis.

The sugar moiety of the deoxypentose nucleic acid was identified tentatively as deoxyribose. The nucleotide composition of the pentose nucleic acid of BCG also was determined.

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