ON THE NUCLEOPROTEINS OF AVIAN TUBERCLE BACILLI*

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The nucleic acids of tubercle bacilli have formed the subject of several studies, among which may be mentioned those by Ruppel (1), Levene (2), and Johnson and his associates (3-5). The work of Menzel and Heidelberger (6) on the fractionation of the proteins of the tubercle bacillus revealed the presence of several protein fractions, rich in phosphorus and in purines, which appeared to be nucleoproteins. The studies of Seibert et al. on tuberculin (7,8) have included experiments on the separation of nucleic acid, present in the crude preparations, from the biologically active protein. The main portion of the nucleic acid preparations studied appears to have belonged to the desoxypentose type; the presence of pentose nucleic acid does not seem to have been recorded.

In connection with work carried out in this laboratory on bacterial glycogen (9) it was observed that borate buffer extracts of ground avian tubercle bacilli contained, in addition to glycogen, a nucleoprotein fraction giving strong color reactions for desoxypentose. This observation provided an opportunity to study a nucleoprotein obtained from the bacterial cells by a mild extraction process at a low temperature that probably suppressed autolytic reactions. Several other disintegration and extraction methods either were unsuccessful or gave inferior results.

The crude nucleoprotein preparations were slightly yellow; they contained a yellow pigment with a blue-green fluorescence and exhibited an absorption peak at 410 m\(\mu\), in addition to the typical ultraviolet spectrum of nucleic acids (Fig. 1). Further fractionation made use of the fact that the principal nucleoprotein fraction was insoluble around pH 4 and could not be precipitated by half saturation with ammonium sulfate. By this procedure a desoxypentose nucleoprotein which contained 3.2 per cent P, and was only slightly contaminated with pentose nucleic acid, could be prepared. The crude preparations, however, contained a much larger proportion of pentose nucleic acid which was removed in the course of the fractionation. The spectra of a crude and of a purified specimen are compared in Fig. 2.

Not much can be said as yet about the nature of the proteins combined

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with the nucleic acids. There is very little likelihood that the crude nucleoprotein fractions contained considerable quantities of a basic protein. Attempts to prepare flavianic acid salts were unsuccessful. Whether the purified deoxypentose nucleoprotein, which amounted to slightly more than one-eighth of the entire preparation, contained a basic protein or a protamine cannot yet be decided.

Our knowledge of the chemistry of nucleoproteins, other than the histone nucleates isolated from various animal cells (10) and the protamine nucleates of fish sperm (11), is as yet very scant. In fact, there exist no generally applicable, safe methods for the isolation of genuine nucleoproteins. Each cellular species will, for the time being, have to be treated as a special case. It should, however, be pointed out that in the isolation methods used in the present study drastic procedures (strong salt solutions, high pH, etc.), which could have produced the cleavage of the conjugated protein, were avoided. It is, for this reason, likely that the isolated nucleoproteins existed as such in the bacterial cell.

Other experiments, e.g. on the disintegration of the nucleoproteins, included in the experimental part, require no special comment. The fact that the treatment of the nucleoprotein at a high electrolyte concentration with a lanthanum salt resulted in the precipitation of the intact nucleoprotein is of interest in showing that in this conjugated protein the phosphoric acid groups of the nucleic acid were available for combination with lanthanum.

The composition of the nucleic acid contained in the nucleoprotein fractions of avian tubercle bacilli, described here, is discussed in the following paper (12).

EXPERIMENTAL

Isolation of Nucleoproteins

Starting Material—The cultivation and isolation of the tubercle bacilli of the avian strain were carried out as described previously (9). The synthetic Sauton medium (13) was employed at pH 7.4 and 38°. The organisms, following washing and drying in the frozen state in a vacuum, contained 4.7 to 4.9 per cent N. Their subsequent handling was made easier by a preliminary extraction with ice-cold peroxide-free ether (20 cc. per gm. of organisms) for 1 day, which resulted in the removal of 4 to 5 per cent of bacillary lipides.

Disintegration of Microorganisms—Numerous attempts to bring about the complete extraction of water-soluble components by methods in which the use of drastic chemical treatment was avoided were not very successful. Among the procedures tried may be mentioned (1) treatment of the bacilli in 0.1 м borate buffer of pH 8.5 with supersonic vibrations; (2) incubation
in the same buffer with crystalline or with commercial trypsin; (3) grinding of the bacilli, mixed with glass powder, at pH 7.5 between rotating glass cones (14), followed by the extraction of the ground material with diethylene glycol. Procedures (1) and (2) were almost completely ineffective; for instance, by the supersonic treatment only 1.1 per cent of the bacterial N was transferred into solution. Procedure (3) had some effect, but no greater effect than the simpler method finally resorted to; the use of diethylene glycol, moreover, complicated the fractionation of the extracted substances.

Preparation of Crude Nucleoprotein—In all following experiments the ether-washed dry tubercle bacilli (mixed with a small amount of the extracting fluid to convert them into a stiff paste) were ground with washed, very fine Pyrex glass powder (diameter 3 μ) in a proportion of 2.5 to 5 parts of glass to 1 part of bacilli.

One typical preparation (Fraction 3, Table I) will be described. A mixture of 25 gm. of bacilli and 100 gm. of glass powder was moistened with borate buffer (pH 8.3) and divided into eight portions, each of which was ground for 30 minutes in a mortar. The ground cells were united, shaken in a refrigerator with 500 cc. of the borate buffer for 2 days, and centrifuged for 30 minutes at 4000 R.P.M. (1900g). The strongly opalescent slightly yellow supernatant was decanted through a filter. The centrifugation residue was washed with 500 cc. of borate buffer which then served for the extraction of a second 25 gm. portion of disintegrated bacilli. In this manner a total of 100 gm. of organisms was processed. The extracts were dialyzed against running water for 48 hours, concentrated by pervaporation to about one-third of the original volume, and again dialyzed against ice-cold distilled water for 72 hours. Ethyl mercurithiosalicylate was added (0.01 percent) and the bacterial glycogen removed by sedimentation at 31,000g (9). The supernatants were once more dialyzed and the crude nucleoprotein fraction was recovered by evaporation of the frozen solution in a vacuum (yield 2.7 gm.). The yields varied for different preparations between 2.4 and 3.4 per cent of the starting material.

Properties of Crude Nucleoprotein—The analytical composition of two preparations is summarized in Table I. All specimens formed light yellow fine fluffs with a silky sheen, which could easily be dispersed in water or buffer solutions. They were not precipitated by the gradual dilution with water of their solution in M sodium chloride. They were precipitable by trichloroacetic acid and gave positive biuret, xanthoproteic, Millon, and Hopkins-Cole reactions. The tests for the presence of desoxypentoses

1 These and most other operations were performed at 4°. The centrifugations were carried out in a refrigerated International centrifuge with multispeed attachment.
TABLE I
Composition of Nucleoprotein Fractions

<table>
<thead>
<tr>
<th>Fraction No.*</th>
<th>Yield</th>
<th>Nitrogen</th>
<th>Phosphorus</th>
<th>Nucleic acid distribution as per cent of total nucleic acid P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per cent</td>
<td>per cent</td>
<td>per cent</td>
<td>Pentose nucleic acid P</td>
</tr>
<tr>
<td>2</td>
<td>2.4</td>
<td>9.1</td>
<td>0.93</td>
<td>41</td>
</tr>
<tr>
<td>3</td>
<td>2.7</td>
<td>10.0</td>
<td>0.72</td>
<td>35</td>
</tr>
<tr>
<td>44</td>
<td>0.4</td>
<td>12.1</td>
<td>3.2</td>
<td>13</td>
</tr>
</tbody>
</table>

* Fractions 2 and 3 represent crude nucleoprotein preparations; Fraction 44 (cf. Table III) is a purified specimen.

Fig. 1. Absorption spectrum of crude nucleoprotein of avian tubercle bacilli (Fraction 2, Table I) in 0.03 M borate buffer of pH 7.9.
with diphenylamine (15) and with cysteine (16) were likewise positive. The substances gave the Feulgen reaction (17); a small shred of the nucleoprotein, heated with $N$ hydrochloric acid for a few seconds, was colored a deep magenta on contact with reduced fuchsin.

Solutions of the crude nucleoprotein fractions were faintly yellow, with a weak blue-green fluorescence which became much stronger in the light of a quartz lamp. The complete spectrum of one preparation is reproduced in Fig. 1. It will be seen that the substance exhibited one weak band in the visible portion of the spectrum, with a center at 410 $\mu m$ (determined at a concentration of 18.6 $\gamma$ of nucleoprotein P per cc.) and one very strong band in the ultraviolet at 259.5 $\mu m$ (3.7 $\gamma$ of P per cc.). Absorption minima were observed at 380 and 243 $\mu m$.

**Fractionation of Nucleoprotein**

*Inhomogeneity of Crude Nucleoprotein—*After the sedimentation of the bacterial glycogen described before, the nucleoproteins moved as a rule with a single sharp boundary in the electrophoresis cell. Occasionally the extracts contained a small faster component, but in practically all cases they were found to be contaminated with an electrophoretically almost immobile fraction, probably residual glycogen. One of the solutions used showed, for instance, the following components (barbiturate buffer of pH 7.8, ascending mobilities): Component I, $-0.34$ (24 per cent of the total); Component II, $-6.9$ (53 per cent); Component III, $-10.6 \times 10^{-5}$ sq. cm. per volt per second (23 per cent). The scarcity of the available material made attempts at fractionation by electrophoresis impracticable and other ways to reach this goal were chosen.

*Influence of pH on Precipitation of Nucleoprotein—*The experiments presented in Table II demonstrated the existence of a relatively narrow range (pH 3 to 4) within which about 83 per cent of the total N and about 70 per cent of the total P were found in the insoluble portion. At higher pH values the precipitation was negligible.

The nucleoprotein was not precipitated by half saturation with ammonium sulfate at pH 6.3. Under these conditions, the insoluble portion (65 per cent of the total) was found to contain N 13.2, P 0.21 per cent; the supernatant yielded a fraction containing N 7.3, P 1.1 per cent. These findings, viz. the flocculation of a nucleoprotein fraction around pH 4, which, however, in contrast to other admixed proteins failed to precipitate in half saturated ammonium sulfate, served for the purification experiments.

*2* The spectroscopic measurements were carried out in the Beckman quartz spectrophotometer. The reasons for the use of $\epsilon(P)$, the atomic extinction coefficient with respect to phosphorus, were discussed previously (18).

*3* We wish to thank Dr. D. H. Moore for the electrophoresis experiments.
Purification of Nucleoprotein—The dialyzed crude nucleoprotein solution (190 cc.), containing a total of 265.1 mg. of N and 16.1 mg. of P, was brought to pH 4.3 by the addition of 2 per cent acetic acid. The mixture in which a precipitate appeared immediately was chilled for 3 hours and centrifuged. The sediment was washed with ice-cold 0.05 M citrate buffer of pH 4.3, dissolved in borate buffer of pH 8.4, and the solution after prolonged dialysis was evaporated in a vacuum in the frozen state. This fraction, listed as Fraction 41 in Table III, formed a slightly yellowish fluff. The supernatants and washings yielded, after dialysis, Fraction 42 (Table III) which, in addition to some protein (almost free of P), contained much glycogen.

<table>
<thead>
<tr>
<th>Experiment No.*</th>
<th>pH</th>
<th>Analysis of supernatant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nitrogen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weight (mg.) Per cent of total</td>
</tr>
<tr>
<td>1</td>
<td>7.2</td>
<td>2.79 100</td>
</tr>
<tr>
<td>2</td>
<td>6.0</td>
<td>2.75 98.6</td>
</tr>
<tr>
<td>3</td>
<td>5.1</td>
<td>2.47 88.5</td>
</tr>
<tr>
<td>4</td>
<td>4.1</td>
<td>0.50 17.9</td>
</tr>
<tr>
<td>5</td>
<td>3.0</td>
<td>0.45 16.1</td>
</tr>
<tr>
<td>6</td>
<td>1.8</td>
<td>1.15 41.2</td>
</tr>
</tbody>
</table>

* Experiment 1 represents the original protein solution in distilled water. The supernatants presented in Experiments 2 to 6 were obtained by the addition of equal amounts of 0.1 M citrate buffers of the indicated pH values and centrifugation, after 20 hours storage at 5°, at 4000 r.p.m.

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Starting material, fraction No.</th>
<th>Nitrogen</th>
<th>Phosphorus</th>
<th>N:P per cent ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm.</td>
<td>per cent</td>
<td>per cent</td>
<td>Proportion of crude nucleoprotein N (Fraction 4)</td>
</tr>
<tr>
<td>4</td>
<td>1.68</td>
<td>13.5</td>
<td>85.6</td>
<td>0.73</td>
</tr>
<tr>
<td>41</td>
<td>0.72</td>
<td>4.6</td>
<td>12.5</td>
<td>0.12</td>
</tr>
<tr>
<td>42</td>
<td>1.36</td>
<td>12.1</td>
<td>62.5</td>
<td>0.20</td>
</tr>
<tr>
<td>43</td>
<td>0.29</td>
<td>12.1</td>
<td>13.3</td>
<td>3.2</td>
</tr>
<tr>
<td>44</td>
<td>(1.67 gm.)</td>
<td>1 (1.67 &quot;&quot;)</td>
<td>13.5</td>
<td>85.6</td>
</tr>
</tbody>
</table>

# Table II

Influence of pH on Precipitation of Nucleoprotein

<table>
<thead>
<tr>
<th>Experiment No.*</th>
<th>pH</th>
<th>Nitrogen</th>
<th>Phosphorus</th>
<th>N:P ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg.</td>
<td>Per cent of total</td>
<td>mg.</td>
</tr>
<tr>
<td>1</td>
<td>7.2</td>
<td>2.79</td>
<td>100</td>
<td>0.170</td>
</tr>
<tr>
<td>2</td>
<td>6.0</td>
<td>2.75</td>
<td>98.6</td>
<td>0.168</td>
</tr>
<tr>
<td>3</td>
<td>5.1</td>
<td>2.47</td>
<td>88.5</td>
<td>0.156</td>
</tr>
<tr>
<td>4</td>
<td>4.1</td>
<td>0.50</td>
<td>17.9</td>
<td>0.060</td>
</tr>
<tr>
<td>5</td>
<td>3.0</td>
<td>0.45</td>
<td>16.1</td>
<td>0.048</td>
</tr>
<tr>
<td>6</td>
<td>1.8</td>
<td>1.15</td>
<td>41.2</td>
<td>0.056</td>
</tr>
</tbody>
</table>
The precipitate produced by the addition of 150 cc. of saturated ammonium sulfate solution to 1.67 gm. of Fraction 41, dissolved in 150 cc. of water, was collected by centrifugation, washed repeatedly with half saturated ammonium sulfate, dissolved in borate buffer of pH 8.2, and dialyzed. This solution yielded Fraction 43 (Table III) as a slightly yellow-

![Fig. 2. Ultraviolet absorption of nucleoproteins in 0.03 M borate buffer of pH 7.9. Curve I, purified nucleoprotein (Fraction 44, Tables I and III). Curve II, crude nucleoprotein (Fraction 2, Table I).]

ish fine powder. The supernatants from the ammonium sulfate precipitation, freed of electrolytes by prolonged dialysis and evaporated in the frozen state in a vacuum, yielded the purified nucleoprotein, Fraction 44 (Table III), which formed a white fiber felt.

As may be gathered from an inspection of Table III, the first purification step, *i.e.* the precipitation at pH 4.3, was accompanied by some loss in
phosphorylated constituents. This probably took place in the course of the dialysis of Fraction 42, as a comparison of the data reported for this fraction in Table III with those for the corresponding preparation in Table II (Experiment 4) will show. The subsequent fractionation with ammonium sulfate, on the other hand, entailed very little loss, 89 and 98 per cent respectively of the N and P contained in Fraction 41 being recovered in Fractions 43 and 44.

Absorption in Ultraviolet—Fig. 2 reproduces the absorption spectrum in the ultraviolet of the purified nucleoprotein, Fraction 44 (Curve I). The solution examined at a concentration of 7.0 γ of P per cc. had a center of absorption at 258 mμ and a minimum at 237 mμ. For purposes of comparison, the detailed spectrum of Fraction 2 (Table I) is likewise given (Curve II). Both nucleoproteins had very similar spectra in the ultraviolet. The fluorescent pigment, however, present in all crude preparations (compare Fig. 1), had disappeared from Fraction 44; it remained for the most part in Fraction 43.

Nucleic Acid Distribution—Since the customary colorimetric methods for the estimation of nucleic acids, based on the behavior of the sugar components of the purine nucleotides, cannot be used in the presence of proteins, the approximate distribution of pentose and desoxypentose nucleic acid phosphorus in crude and purified preparations was determined by the method of Schmidt and Thannhauser (19). We are very grateful to Dr. G. Schmidt of Tufts College Medical School for the estimations included in Table I. It will be noted that most of the pentose nucleic acid apparently remained in Fractions 42 and 43 (Table III) and that the purified nucleoprotein, Fraction 44, contained, for the most part, desoxypentose nucleic acid.

Viscosity—The viscosity of solutions of the nucleoproteins in 0.1 м borate buffer of pH 7.9 was determined at 37.0° in Ostwald-Fenske pipettes. The values are expressed here as the specific viscosity: \( \eta_{sp} = \frac{\eta}{\eta_0} - 1 \), where \( \eta \) and \( \eta_0 \) are the viscosities of the solution and the solvent respectively. The substances exhibited a low viscosity. A 0.28 per cent solution of Fraction 2 (Table I) had \( \eta_{sp} = 0.14 \); the purified compound Fraction 44 (Tables I and III) had, in a 0.1 per cent solution, \( \eta_{sp} = 0.28 \).

Disintegration Experiments; Isolation of Nucleic Acids

Effect of Proteolytic Enzymes—The effect of crystalline preparations of trypsin and chymotrypsin on several nucleoprotein samples was studied viscometrically at pH 7.9 and 37°. No increase in viscosity was observed. The degradation of the protein moiety by enzymatic action would have been expected to bring about an increase in viscosity owing to the release of nucleic acid (20, 21).
Disintegration Attempts—Experiments aiming at the precipitation of the protein moiety as a salt with flavianic acid were unsuccessful, nor could the presence of a basic protein of the protamine or histone types be demonstrated in other ways. Prolonged dialysis against M sodium chloride, a procedure described as productive of splitting in the case of a nucleoprotein (11), proved ineffective. A solution of 103.6 mg. of Fraction 3 (Table I) in 10 cc. of M sodium chloride was subjected to dialysis against a salt solution of the same concentration at 4° for 8 days. The solution, after being freed of salt, yielded 73.2 mg. of a substance with completely unchanged analytical values; viz., N 9.9, P 0.70 per cent.4

Effect of Lanthanum—A solution of 370 mg. of the crude nucleoprotein, Fraction 3 (Table I), in 20 cc. of a sodium chloride-borate buffer mixture of pH 7.7 (0.08 M with respect to borate, 1.2 M with respect to NaCl) was clarified by centrifugation at 4800 r.p.m. The gelatinous precipitate produced by the addition to the supernatant of 5 cc. of 2.5 per cent aqueous lanthanum nitrate was removed by centrifugation after being chilled overnight, washed repeatedly with the borate-NaCl mixture, and then extracted with 0.1 M acetate buffer of pH 4.9. The insoluble extraction residue, exhaustively washed with distilled water, was suspended in water, and this suspension, as well as the dialyzed supernatants (obtained at pH 7.7 and 4.9), was evaporated in a vacuum in the frozen state. The results, summarized in Table IV, show an interesting parallelism with the fractionation experiments described above. The portion not precipitated by lanthanum (Fraction 31, Table IV) appears quite similar to Fraction 42 in Table III, the major part consisting of material free of P and N, probably glycogen. The entire nucleoprotein, on the other hand, combined with lanthanum and became insoluble at pH 7.7. No indication of a dissociation into nucleic acid and protein in the presence of a high

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Properties</th>
<th>Weight</th>
<th>Nitrogen</th>
<th>Phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg.</td>
<td>per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>31</td>
<td>Soluble at pH 7.7</td>
<td>82.6</td>
<td>4.5</td>
<td>10.0</td>
</tr>
<tr>
<td>32</td>
<td>&quot; &quot; &quot; 4.9</td>
<td>21.3</td>
<td>13.5</td>
<td>7.8</td>
</tr>
<tr>
<td>33</td>
<td>Insoluble at pH 7.7 and 4.9</td>
<td>157.1</td>
<td>14.1</td>
<td>59.9</td>
</tr>
</tbody>
</table>

4 The small amount of yellow pigment which, as was mentioned above, accompanies the crude nucleoprotein preparations was not removed by the dialysis.
salt concentration was observed. These experiments indicated that the phosphoric acid groups of the nucleic acid were not involved in the combination with protein in such a manner as to make them unavailable for salt formation with lanthanum.

Action of Saturated Sodium Chloride Solution—The efficacy of saturated sodium chloride solutions in the cleavage of the nucleic acid-histone bond is well known (22, 23). A solution of 206.5 mg. of Fraction 3 (Table I) in 12 cc. of 10 per cent sodium chloride was saturated with NaCl, after which turbidity and precipitation occurred. The chilled mixture was centrifuged and 2 volumes of alcohol were added to the supernatant. The precipitate thus produced was not all soluble in water and physiological saline. The united insoluble residues were suspended in 0.1 M borate buffer of pH 8.4, subjected to prolonged dialysis, and recovered in the usual manner. The insoluble protein fraction weighed 102.7 mg. (35 per cent of the starting material), formed an almost white, light fluff, and contained N 13.8, P 0.4 per cent. The saline solution was freed of residual protein by five treatments for 16 hours each with 9:1 chloroform-octanol (24) and the crude nucleic acid precipitated by the addition of 4 volumes of alcohol. Reprecipitation with 1 volume of alcohol finally removed the yellow pigment accompanying the crude nucleoprotein. The nucleic acid preparation obtained in this manner weighed 12.7 mg. and formed white fibers which gave quite viscous solutions. The analytical figures (N 8.2, P 4.8 per cent) showed it to be still contaminated with bacterial glycogen. It could be further purified by the procedures discussed in the following paper (12).

These experiments indicated that the cleavage of the nucleoprotein by saturated salt solutions was not complete: 21 per cent of the original P remained in the precipitated protein fraction, 28 per cent was recovered in the liberated nucleic acid.

Action of Sodium Desoxycholate—This very active detergent could be utilized for the splitting of the nucleoprotein (cf. (25)). A solution of 820 mg. of Fraction 3 (Table I) in 40 cc. of physiological saline was mixed with an equal volume of 1 per cent aqueous sodium desoxycholate. The mixture (pH 7.6) was chilled overnight and the precipitate produced by the addition of 3 volumes of absolute alcohol removed by centrifugation. It was washed with alcohol and its solution in 30 cc. of saline was shaken with 20 cc. of chloroform-octanol for 20 hours. The coagulated protein portion was recovered, washed with water, 70 and 100 per cent ethyl alcohol, and ether, and dried. It weighed 208 mg. and contained N 14.1, P 0.20 per cent (7 per cent of the original P). The treatment of the saline solution with chloroform-octanol was repeated four times for a total of 90 hours. The nucleic acid was twice precipitated from its solution in saline by the addition of 4 volumes of alcohol and subsequently three times with
1 volume of alcohol. The yellow, blue-fluorescing pigment mentioned before was soluble in 50 per cent alcohol and was thus removed. The nucleic acid, precipitating as a coherent bundle of slimy fibers, was again dissolved in 12 cc. of saline, and the solution was clarified by centrifugation at 11,000g for 30 minutes, dialyzed against tap and ice-cold distilled water for a total of 4 days, and evaporated to dryness in the frozen state in vacuo. The nucleic acid, 67.2 mg. of white fibers, contained N 10.6, P 6.3 per cent (72 per cent of the original P) and was further purified (12).

Similar nucleic acid preparations could be obtained by the direct extraction of dry ether-washed tubercle bacilli with 0.5 per cent sodium desoxycholate solution, but the yields were smaller and the products more contaminated with polysaccharides.

Purification of Nucleic Acids—As pointed out repeatedly, the main obstacle to the preparation of pure undegraded nucleic acids from the bacillary nucleoproteins lay in the difficulty with which contaminating glycogen and other substances were removed. The low percentage values for nitrogen and phosphorus indicated the presence of non-nitrogenous impurities. Purification by electrophoresis proved possible, although it was accompanied by considerable losses. One example will be given here. A crude nucleic acid sample, prepared by extraction of the bacilli with 0.5 per cent sodium desoxycholate, was found to contain P 3.8 per cent (yield 30.7 mg. from 37 gm. of ether-washed bacilli). The entire quantity was dissolved in 2 cc. of barbiturate buffer of pH 7.8 and subjected to electrophoresis in a micro cell. The examination revealed three components with the following descending mobilities: Component I, -1.6; Component II, -10.9; Component III, $-15.2 \times 10^{-5}$ sq. cm. per volt per second. Components II and III were removed separately, subjected to dialysis, and recovered by evaporation in the frozen state in vacuo. Component II weighed 9.0 mg. and contained P 1.9 per cent (15 per cent of the original P). Component III was nucleic acid; it consisted of white fibers weighing 6.6 mg. and contained N 12.9, P 7.6 per cent (43 per cent of the original P).

SUMMARY

The properties and the purification of a nucleoprotein fraction isolated from avian tubercle bacilli (containing desoxypentose and pentose nucleic acids) are discussed. Methods for the cleavage of the nucleoprotein and the isolation of desoxypentose nucleic acid are reported.

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