AN ELECTROPHORETIC EXAMINATION OF CELL-FREE EXTRACTS FROM VARIOUS SEROLOGICAL TYPES OF GROUP A HEMOLYTIC STREPTOCOCCI

by

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INTRODUCTION

Knowledge of the protein composition of the bacterial cell is fragmentary; particularly so with respect to the group A streptococci. SEVAG, SMOLENS AND LACKMAN[1] have reported the nucleic acid content of Group A streptococci to vary from 15 to 25% of the cellular extract. HEIDELBERGER AND KENDALL[2] as early as 1931 isolated a labile nucleoprotein from streptococci. More recently SCHACHMAN, PARDEE AND STANIER[3] examined in the ultracentrifuge extracts from various genera of bacteria, prepared by several extraction procedures. These workers observed that the sedimentation behavior of components present in these extracts were similar, irrespective of the manner of extraction. WEIBULL[4] also examined, in the ultracentrifuge, extracts of *Bacillus megaterium* prepared by several procedures. WEIBULL found three major peaks in the sedimentation patterns of these extracts. The sedimentation coefficients calculated for these three peaks were in agreement with those reported by SCHACHMAN, PARDEE AND STANIER[3]. BERRIDGE AND BRIGGS[5] have shown that different electrophoretic patterns were obtained from extracts prepared from streptococci belonging to 5 serological groups.

In earlier studies in this laboratory it was observed that cell free extracts of streptococci prepared by shaking and by sonic oscillation were water-soluble and remained so after being dialyzed and dried from the frozen state. The use of such extracts in enzymic work by SLADE[6] and in the isolation of antigenic components of the cell by HAHN AND SLADE[7] made it of interest to inquire further into the chemical and physical nature of these extracts.

This report describes the results of electrophoretic examinations of extracts from six serological types of Group A hemolytic streptococci.

EXPERIMENTAL

Culture of organisms

The broth used for the culture of both inoculum and cell mass was as follows: 37 g brain heart infusion (Difco) was dissolved in 800 ml water and autoclaved. To this was added 200 ml of solution (sterilized by filtration) containing 18 g glucose, 3 g NaCl, 8 g $NaHCO_3$, 1.5 g Na_2HPO_4. The final pH was 7.2–7.5.

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The inoculum was grown for 5 h in 10 ml broth at 37° C and 9 ml used to inoculate 1 liter. The flasks were held at 37° C for 18 h and the cells harvested by centrifugation. The cell paste was washed 3 times with distilled water and suspended in water to a concentration of 30% (wet weight).

Preparation of extracts: Sonic oscillation

Cell suspensions (15–20 ml) were treated in a Raytheon 9 kc oscillator for 3 h at plate voltage 130. The temperature in the chamber was maintained at 2° C by circulation of an alcohol-water solution during the process. After treatment the solution was centrifuged at 5° C for ½ h at 20,000 *g*, dialyzed against water for 18 h and lyophylized. The non-extractable material was in most cases suspended and dried.

Preparation of extracts: Shaking

Cell suspensions (5 ml/cup) were shaken for 30 min with 5 g glass beads* at 5° C in a Mickle tissue disintegrator**. The preparation was centrifuged ½ h at 20,000 *g* and the supernatant drawn off. The cell debris–bead mixture was washed with 2 ml water and re-centrifuged. The wash water was combined with the original extract. Total volume was approximately 6 ml. The extract was dialyzed 18 h against water and lyophylized.

Electrophoretic analysis

Determinations were made in a conventional Tiselius assembly equipped with a Philpot-Svensson schlieren optical system[8,9]. The experiments were performed in an all glass cell assembly at 0.5° C. Conductivities were measured at 0.0° C; the mobility values therefore obtain at 0.0° C[10]. The conductivity of the buffer was used for mobility calculations. The values reported were calculated from tracings of enlargements of the descending patterns in the usual manner[11,12]. All experiments were made in veronal buffer at an ionic strength (μ) equal to 0.10 and a pH 8.55 ± 0.05. The composition and conductivity of this buffer as used in this laboratory has been reported[13]. Solutions were prepared by dissolving 200 mg of the dry cell extract in 15 ml of buffer and dialyzing overnight against buffer. All patterns shown are photographs of the descending limb of the cell after 120 minutes under a potential gradient of 6.4 volts cm^{-1}. Ascending patterns are similar but not enantiographic. Patterns obtained from non-dialyzed solutions or from solutions dialyzed 72 h were indistinguishable from those prepared as indicated above. The solutions were clarified by centrifuging at 10,000 *g* for 10 min prior to placing them in the cell. A trace of precipitate was usually found.

RESULTS

In Fig. 1 are shown typical patterns obtained from extracts of four organisms differing in serological type. In Fig. 2 a tracing of the general pattern can be seen along with the mobilities of the components arbitrarily drawn in as shown. A minimum of at least eleven components are present in the extract.

The resolution is insufficient to make worthwhile a calculation of relative composition, especially when nothing is known about the refractive indices of the components. In Table I are listed the various organisms which were examined. In Table II the relative amount of each component from each extract was compared with an arbitrarily selected pattern (from strain H 713, Type 4) and indicated as greater than (+) or less than (—) this standard. This is the same pattern shown in Fig. 1b and traced in Fig. 2. This pattern was selected primilarly because it was the only organism displaying a qualitative difference in composition. Component 2 (Fig. 2) as found only in this organism. Also included in the Table II are the yields of dry extract obtained in each instance.

It should be emphasized that such comparisons are valid only when the extract is prepared in a stereotyped manner. Changes in the duration of sonic oscillation were found to alter the relative amounts of the various components. On the other hand several extracts prepared in an identical fashion from the same culture were found to give reproducible electrophoretic patterns.

* Dr. V. A. KNIVETT of the Chemical Research Laboratory, Teddington, Middlesex, England kindly gave us the ballotini beads (0.003" dia.) used in this investigation.

** Manufactured by H. Mickle, Hampton, Middlesex, England.

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The ultraviolet absorption pattern given by a typical extract is shown in Fig. 3. The ratio of optical density readings at 280 mμ to 260 mμ is a measure of the nucleic acid content of the mixture. These ratios are listed in Table II as well as the nucleic acid content determined independently by the SCHNEIDER method[14].

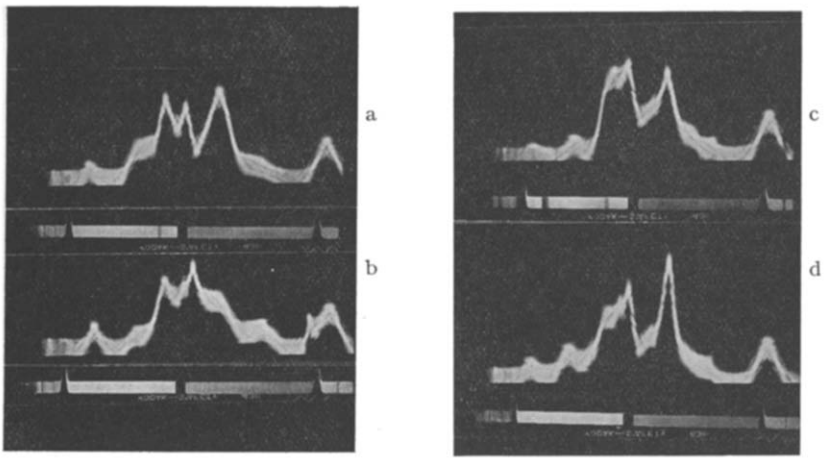

Fig. 1. Electrophoretic patterns (descending) of sonic extracts of various type specific streptococci after 120 minutes under a potential gradient of 6.4 volt cm^{-1} in veronal buffer μ = 0.10 pH 8.6, protein concentration 1.3% by weight, diagonal slit setting 60°; a. SF 42 (type 12); b. H 713 (type 4); c. N 19 (type 19); d. Blackmore (type 11).

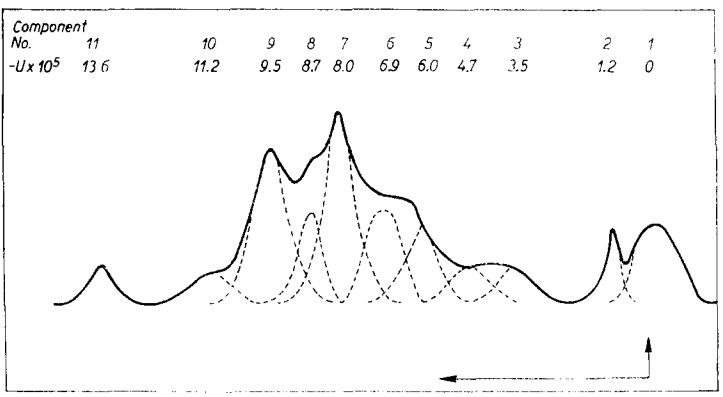

Fig. 2. Tracing of the descending electrophoresis pattern of an extract of H 713 (type 4) streptococcus obtained by sonic oscillation. The arbitrary selection of the eleven components is shown with the corresponding mobilities (μ) in pH 8.6 μ 0.10 veronal buffer. Pattern after 120 minutes at 6.4 volt cm^{-1}.

In Fig. 4 the electrophoretic pattern of an extract prepared by sonic oscillation is compared with the pattern of a Mickle extract. These two extracts were obtained from the same culture. It is obvious that although the same components are present in each extract the relative proportions of the components are quite different. The sonic oscillation method provided a greater amount of soluble material from the same organism when equal amounts of cells were used for extraction. On the other hand, the

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TABLE I

SEROLOGICAL TYPE AND SOURCE OF CULTURES EXAMINED

Culture	*Type*	*Source*	*Origin of culture*
Richards	3	Dr. E. W. TODD*	Puerperal fever
Blackmore	11	Dr. E. W. TODD*	Scarlet fever
H 713	4	Dr. E. W. TODD*	—
SF 42	12	Dr. E. W. TODD*	Scarlet fever
S 43	6	Dr. R. LANCEFIELD	Broncho-pneumonia
N 19	19	Dr. A. F. COBURN	Scarlet fever
T 19	19	Dr. JOHN SEAL	Tonsillitis
B 19	19	Dr. JOHN SEAL	—
2884 F	19	Dr. A. T. WILSON	—
12 RN	12	Dr. C. H. RAMMELKAMP, Jr.	Glomerulonephritis

* Deceased

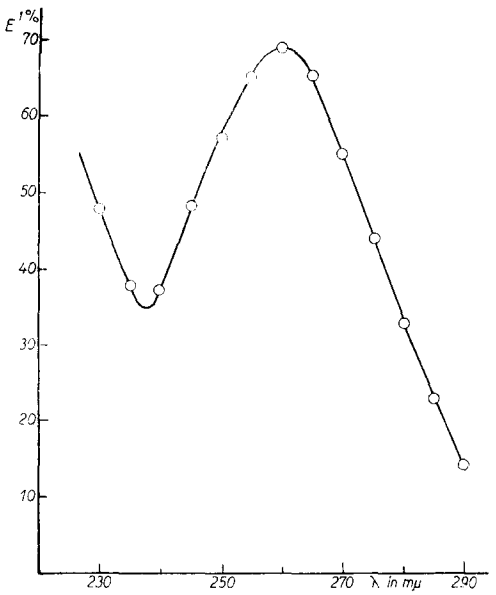

Fig. 3. The ultraviolet extinction curve of the extract prepared by sonic oscillation of 12 RN. Total concentration 0.005% in water. The ordinate is $E^{1\%} = 1/C \log I_0/I$ where C is the concentration in g per 100 ml. The abscissa is the wavelength in millimicrons.

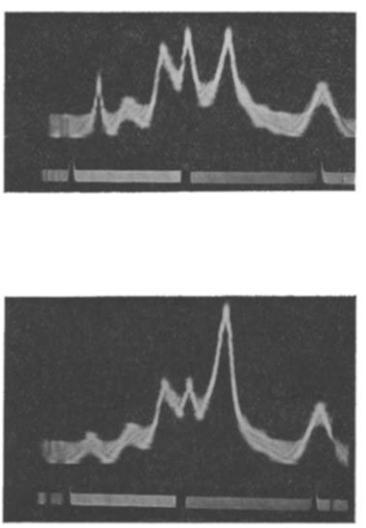

Fig. 4. Comparison of the electrophoretic patterns obtained on extracts of S43 (type 6) prepared by two different methods; top picture—shaking with ballotini beads, lower picture—sonic oscillation. The cells used here were obtained from a single culture.

percent nucleic acid in the extract has been found to be somewhat higher when the shaking method was employed as can be seen in Table II.

A total of 15 extracts prepared by sonic oscillation and 4 prepared by shaking were analysed. Of the extracts prepared by sonic oscillation, B19 was found to contain the least amount of nucleic acid (15%) and 12RN was found to contain the greatest amount (31%). In Fig. 5 the electrophoretic patterns are compared. In Fig. 5a the pattern obtained from B19 shows a very large amount of component 4 and is low in components

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TABLE II

CHEMICAL AND PHYSICAL ANALYTICAL DATA ON GROUP A HEMOLYTIC STREPTOCOCCI

Strain	Serological type	% extracted	Method of extraction	$\frac{OD_{280}}{OD_{260}}$	% nucleic acid	Component 1	2	3	4	5	6	7	8	9	10	11
H 713	4	42	S	0.55												
4 RM	4	63	S	0.53	29											
4 RM	4	44	M	0.52	31											
S 43	6	48	S	0.59	20											
S 43	6	58	S	0.60	19											
S 43	6	33	M	0.59	21											
Richards	3		S	0.53	29											
Blackmore	11		S	0.55												
Blackmore	11	58	S	0.58												
Blackmore	11	40	S	0.55												
SF 42	12	44	S	0.54	26											
12 RN	12	53	S	0.52	31											
12 RN	12	45	M	0.51	32											
N 19	19		S	0.57	20											
N 19	19	34	S													
T 19	19	26	S	0.60	16											
B 19	19	34	S	0.61	15											
2884 F	19	37	S	0.56	25											
2884 F	19		M	0.54	27											

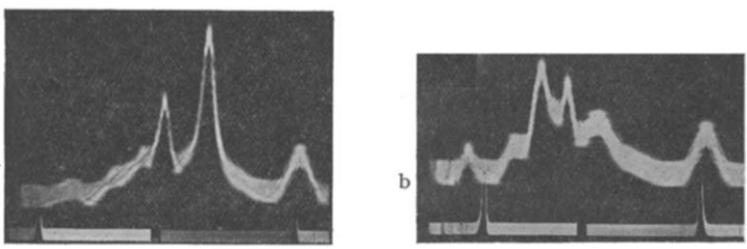

Fig. 5. Comparison of electrophoretic patterns obtained from (a) B19 containing 15% nucleic acid in the extract and (b) 12RN containing 31% nucleic acid in the extract. Both extracts prepared by sonic oscillation.

8, 9, 10. In Fig. 5b the pattern obtained from 12RN is almost devoid of component 4 and shows a large amount of component 9 with relatively large amounts of components 7, 8 and 10. It can be concluded from the above observations that it is unlikely that component 4 contains nucleic acid and that components 7, 8, 9 and 10 are probably nucleoproteins. Component 9 almost certainly is a nucleoprotein. The above conclusion is supported by a similar correlation between the nucleic acid content of the extract and the electrophoretic pattern in every case examined. The above conclusion is consistent, furthermore, with the mobilities of the components.

DISCUSSION

Examination of the data listed in Table II discloses several things. First, there is the remarkable uniformity of the quantity of component 1, irrespective of the percent of extractable material. Second, component 2 was found only in the type 4 organism,

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H713. Third, in instances where the shaking method was used to extract the organism, the percent of nucleic acid in the extract is greater than in comparable extracts prepared by sonic oscillation. As can be seen in Table II, in these instances, component 11 was correspondingly elevated. Component 11 has the mobility of free nucleic acid. However, as can be seen in Fig. 1, 2, 4 and 5, the total amount of this component is relatively small. Relatively little free nucleic acid is therefore present in these extracts. This does not necessarily mean that there is little free nucleic acid in the cell, however. It is possible that free nucleic acid complexes with other components in the extract at pH 8.6 in μ0.10 veronal buffer. Variability in the amount of other components was great, however, and the amount of component 11 was quite uniform in all the extracts. The former interpretation, therefore, is the more likely one. The small amount of free nucleic acid present in these extracts is evidence that no appreciable release of nucleic acid from nucleoprotein by the action of nucleases occurred during the extraction and dialysis steps.

An attempt to correlate low yields of extract with lower percentage of nucleic acid in the extract was unsuccessful. In general, the type 19 organisms yielded the smallest amount of extract. Comparison of the two type 19 organisms, strain 2884F and B19, showed that although the percent of extract was nearly the same, the amount of nucleic acid in the extract was entirely different. In this connection in strain S43 which yielded a high percent of extract, the percent nucleic acid in the extract was found to be low.

The salt boundaries were greater than theoretical[15] and in part, at least, probably represent components possessing essentially zero charge at pH 8.6. Similar large δ and ε boundaries were present in extracts run in acetate buffer at pH 4.7. Large salt boundaries were also present in the preparation of BERRIDGE AND BRIGGS[5]. In view of the large amount of carbohydrate present in the bacterial cell[16], it would appear probable that the components in these salt boundaries are essentially uncharged polysaccharides.

The possibility that extraction by sonic oscillation may produce changes in the structure and state of aggregation of the cellular components can not be overlooked. With regard to this possibility it is of interest that SCHACHMAN, PARDEE, AND STANIER[3] and WEIBULL[4] found that the sedimentation coefficients of components present in bacterial extracts were reproducible irrespective of the method of extraction. The reproducibility of the electrophoretic patterns we have obtained in repeat experiments with the same organism suggests that if such changes have occurred they were not random in nature. As mentioned above, it was observed that variation in time of sonic oscillation altered the relative amounts of certain components. The significance of this observation is not immediately apparent.

Comparison of the electrophoretic patterns obtained from sonic extracts with those obtained by shaking tends to support the conclusion that intramolecular chemical bonds were not ruptured by sonic oscillation. (See Fig. 4.) In this instance, a cell suspension was divided into two parts; one part was extracted by sonic oscillation, the other part by the shaking. The yield of water soluble extractables was found to be consistently higher from the oscillation method of extraction. All of the components present in one extract, however, were observed in the other extract. The ratio of component 4 to components 7, 8, 9 and 10 was found always to be greater in the extract prepared from the sonic oscillation method.

It would appear that the sonic oscillation method is the more efficient one for

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solubilizing component 4. Another possibility, in view of the lower yield of total extract, is that component 4 is rendered insoluble by the shaking method in which case the insoluble portion of component 4 is found in the nonextractables. Irrespective of the above interpretation, inasmuch as components with the same mobility were present using either method of extraction, it seems probable that these components represent separate and distinct units in the composition of the cell.

A necessary conclusion is that different serological types of Group A streptococci exhibit fundamental differences in the extractibility of their components or, that there must be quantitative differences in composition of the organisms.

Sonic extracts of four type 19 cultures (N19, B19, T19, 2884F) were compared. The electrophoretic patterns could be distinguished on a quantitative basis, particularly with respect to components 5, 6, 7, 8 and 9. Qualitatively each extract gave the same pattern.

It seems significant that again the high percentage of nucleic acid in the extract obtained from 2884F could be correlated with larger amounts of components 7, 8 and 9. On the other hand, the amount of free nucleic acid (component 11) was similar in all four extracts.

It is of interest that the mobilities of half of the components in the bacterial extracts were greater than those of animal blood serum proteins when compared in the same buffer system. The nucleic acid content of the extracts vary from 15–32% and can be associated with the components of higher mobility, as mentioned above. It is noteworthy that these bacterial proteins were water soluble both before and after lyophilizing. This property makes it feasible to prepare and stockpile considerable amounts of such extracts in preparation for separation and characterization of the individual components.

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SUMMARY

1. An electrophoretic examination of water soluble extracts of six serological types of Group A hemolytic streptococci has been made. Extracts were prepared from aqueous cell suspensions by (a) sonic oscillation and (b) shaking with glass spheres in a Mickle tissue disintegrator.

2. The same components were found with both methods of extraction, although the proportions of the various components were different. The sonic oscillation method of extraction under the conditions used was the more efficient.

3. Comparison of the electrophoretic patterns of six serological types and four strains of a single type using the sonic oscillation method of extraction showed marked and distinctive differences in composition. Duplication of patterns was observed when a single strain was extracted.

4. It was concluded that different serological type Group A hemolytic streptococci exhibit fundamental differences in the extractability of their component parts and possibly in their quantitative composition.

5. Components with mobilities in the range $7-11 \cdot 10^{-5}$ cm^2 sec^{-1} $volt^{-1}$ could be associated with the nucleic acid content of the extract and are probably nucleoproteins.

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RÉSUMÉ

1. Des extraits aqueux de six types sérologiques de streptocoques hémolytiques du groupe A ont été soumis à l'électrophorèse. Les extraits ont été préparés à partir de suspensions de cellules dans l'eau (a) par oscillations soniques et (b) par agitation en présence de billes de verre dans un désintégrateur de tissu Mickle.

2. Les mêmes constituants ont été trouvés quelque soit la méthode d'extraction, mais dans des proportions différentes. La méthode aux oscillations soniques est la plus efficace dans les conditions employées.

3. La comparaison des diagrammes d'électrophorèse des six types sérologiques et de quatre souches d'un même type, extraits par la méthode aux oscillations soniques, révèle des différences profondes et spécifiques de composition. Les diagrammes obtenus avec une seule souche sont reproductibles.

4. On peut en conclure que différents types sérologiques de streptocoques hémolytiques du groupe A présentent des différences fondamentales dans l'extractibilité de leurs constituants et peut-être dans leur composition quantitative.

5. Les constituants dont les mobilités sont comprises entre 7 et $11 \cdot 10^{-5}$ $cm^2\ sec^{-1}\ volt^{-1}$ peuvent être associés avec la fraction nucléique des extraits et sont probablement des nucléoprotéines.

ZUSAMMENFASSUNG

1. Es wurden die wasserlöslichen Extrakte von sechs serologischen Typen der hämolytischen Streptococcen 'Gruppe A' elektrophoretisch untersucht. Die Extrakte wurden aus wässrigen Zellsuspensionen hergestellt (a) durch Beschallung und (b) durch Schütteln mit Glaskugeln in einem Mickle Gewebezerstörer.

2. Es wurde mit beiden Extraktionsmethoden dieselben Komponenten gefunden, obwohl die Proportionen der verschiedenen Komponenten variierten. Die Beschallungsmethode der Extraktion war unter den benutzten Bedingungen wirksamer.

3. Wenn mit der Beschallungsmethode extrahiert worden war, zeigte der Vergleich der elektrophoretischen Banden von sechs serologischen Typen und von vier Stämmen desselben Typus markante und unterscheidbare Differenzen in der Zusammensetzung. Doch wurden die gleichen Banden erhalten, wenn ein einziger Stamm extrahiert worden war.

4. Es wird geschlossen, dass jede verschieden serologische Type der hämolytischen Streptococcen 'Gruppe A' fundamentale Differenzen zeigt in der Extrahierbarkeit seiner Teilkomponenten und (damit) möglicherweise seiner quantitativen Zusammensetzung.

5. Komponenten mit Beweglichkeiten im Bereich von 7 bis $11 \cdot 10^{-5}$ $cm^2\ sec^{-1}$ Volt^{-1} konnten in Verbindung gebracht werden mit der im Extrakt enthaltenen Nukleinsäure und sind wahrscheinlich Nukleoproteine.

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