Technical Papers

Infrared Spectra of Pneumococcal Polysaccharides[1]

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It has been demonstrated that dried films of bacteria of various genera, species, and even types have characteristic infrared absorption spectra (*1*). In an effort to correlate the spectral differences with cytochemical structures, we have reported studies of the spectra of various extracts of bacteria, as well as the effect of the whole cell spectrum of certain extraction procedures (*2*).

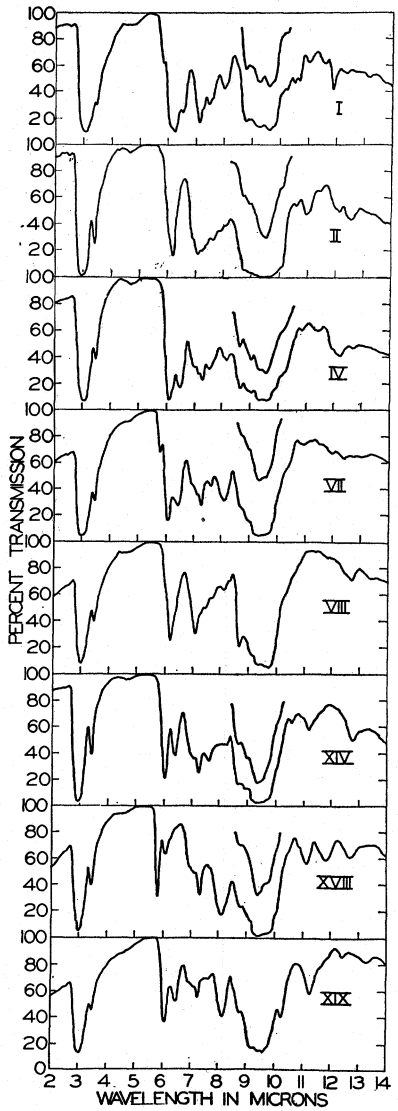

FIG. 1. Infrared spectra of pneumococcal polysaccharides. Types I, II, IV, and XIV were obtained from Dr. Brown and Types VII, VIII, XVIII, and XIX from Dr. Palmer.

[1] We are indebted to Rachel Brown, of the New York State Department of Health, and to John W. Palmer, of E. R. Squibb & Sons, for the polysaccharides used in this study.

Spectra of purified pneumococcal polysaccharides appear to be of sufficient interest to warrant a preliminary report. These substances are of especial interest since they determine the serological type specificity of the whole cell and have been reasonably well characterized as to chemical structure.

Samples of the polysaccharides were prepared by evaporating aqueous solutions to dryness on the surface of silver chloride plates. By constructing a holder for very small silver chloride plates to be used in the microcell adapter of the Perkin-Elmer double-beam recording spectrophotometer, satisfactory spectra were obtained from 0.4 mg. A somewhat thinner film was useful to bring out more detail between 8.5 and 10 μ where the absorption is very strong. The spectrum from 2 to 15 μ can be automatically recorded in 40 min.

The spectra of some of the pneumococcal polysaccharides are shown in Fig. 1. The spectra of Types I, IV, VII, XIV, XVIII, and XIX are quite distinctive and easily identified. On the other hand, the spectra of Types II, III, and VIII are similar, although definitely distinguishable. These spectral relationships are in accord with the analytical data reported in the literature (*3*) which indicate that Types II, III, and VIII are chemically similar in having a low content of nitrogen, phosphorus, and acetyl groups and in giving positive tests for glucose and uronic acid.

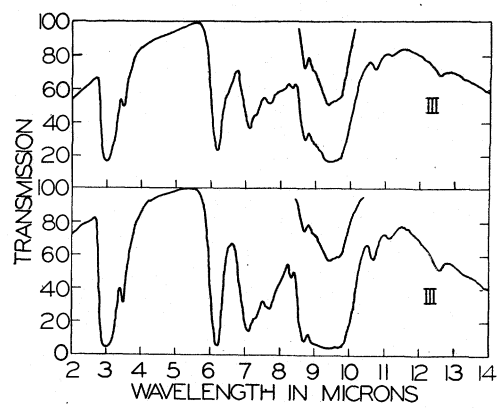

FIG. 2. Infrared spectra of Type III pneumococcal polysaccharides obtained from Dr. Palmer (upper spectrum) and Dr. Brown (lower spectrum).

Spectra of two samples of Type III polysaccharide obtained from different sources and prepared by different methods are shown in Fig. 2. Small differences are present, but these are of a lesser order of magnitude than the differences between Type III and any other type.

These preliminary results indicate some of the uses

of infrared spectrophotometry in immunochemistry: (1) It affords a single, rapid physical test, requiring a small sample of the purified material, which permits the identification of the type-specific polysaccharides. (2) By comparison with standard samples, it may be used as a criterion of purity. (3) Comparison of the spectra of different types provides information on chemical structure and relationships.

A more complete report on this work will be published elsewhere.

References

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The Occurrence of Lymphocytes with Bilobed Nuclei in Cyclotron Personnel[1]

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A preliminary report of observations on the blood of cyclotron workers at the 130″ cyclotron at the University of Rochester called attention to an increased incidence of lymphocytes with bilobed nuclei in cyclotron personnel during the early period of operation of the cyclotron (*1*). It has recently been possible to review the data representing the period extending from 1948, when the initial crew was being formally assembled, through 1951. This review is the subject of the present report. Interest in this subject has been sustained by experimental confirmation of the relationship between an increased number of lymphocytes with bilobed nuclei and exposure to very small amounts of radiation from the cyclotron (*2*).

Hematological monitoring was instituted as a part of the health program for cyclotron personnel in July 1948. The institution of this program prior to the completion of the cyclotron presented a distinct advantage in evaluating the significance of subsequent observations since it permits quantitative comparison of data for the same individuals collected during the control and working periods, as well as comparison of control data for various groups of workers. Throughout the period of cyclotron operation all new employees have had duplicate or triplicate blood studies within 7–10 days after hire, and this has resulted in an ever-expanding body of control data. In most instances, RBC and WBC counts have been done in duplicate, and differential leukocyte counts are based upon examination of 200 leukocytes on a Wright's stained smear. Hemoglobin determinations and platelet counts were done by standard procedures. Throughout the program, particular emphasis has been placed on the cytology of peripheral blood. The basic procedure has been extensive study of good coverslip blood smears of capillary blood. Peroxidase staining was introduced as a routine procedure after the first few cells which appeared to be binucleate lymphocytes were noted. Peroxidase-stained smears are examined from edge to edge, the leukocytes in all good areas counted, and the number and position of all lymphocytes with bilobed nuclei noted. Each cell is subsequently "edited" by the entire group concerned with this study in order to verify the classification. Currently three pairs of peroxidase-stained smears (approximately 8000 leukocytes) are examined for each new employee.

In addition to hematological studies, a complete medical history, as well as the results of physical examinations, is recorded in considerable detail for each individual at the time of hire. A cordial relationship between the cyclotron personnel and the Health Service group facilitated evaluation of the general health of the various individuals during the period covered in this report.

The first evidence of an increased incidence of the abnormal lymphocytes in cyclotron workers was provided by two incidents which occurred during the early days of cyclotron operation. In the first, four physicists were in the cyclotron building when the man at the control panel made certain adjustments that resulted in the possibility that the physicists might have had some slight exposure to the beam.[3] Blood studies were done daily on these four men during the following two weeks and, for the first time in our experience, the presence of several lymphocytes with bilobed nuclei was noted.

Shortly after this, construction of an earth and concrete dike between the cyclotron building and the cyclotron laboratory was begun. This was nearing completion in March 1949, when the second incident occurred. While the cyclotron was running, two machinists started out the back door of the shop in the laboratory building toward the building housing the cyclotron. The men had reached the far end of the dike about 50 feet from the cyclotron building before they realized their mistake and hastily retraced their steps. It was estimated that they were outside the shop only a few minutes.

The two men developed slight, transient leukopenia during the week following exposure, and showed a definite increase in lymphocytes with bilobed nuclei for about three weeks after exposure. Seven control

[1] This paper is based on work performed under contract with the U. S. Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, N. Y.

[2] The authors wish to express their appreciation for the unusually generous cooperation of S. W. Barnes, and the entire cyclotron crew in carrying out the studies on which this report is based.

[3] Both incidents occurred prior to the installation of the various mechanical safety devices which have long since made it virtually impossible for anyone to be in the cyclotron building or the fenced-in cyclotron area while the cyclotron is running.