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The nitrogen content of the pneumococcus: A preliminary report.

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A study of the nitrogen content of various bacterial groups and subgroups was undertaken by the writers some months ago. The object was to determine, if possible, the relationship of the nitrogen content which might occur between members of a single group, and also that between the subgroups of a species where such are known to exist. While progress has been made in this study, much remains to be done, and we are continuing the investigation. A preliminary report of the results of our analyses made upon the pneumococcus, including the four serological subgroups, may prove to be of interest to others. We therefore submit some of the data, although the study is not completed.

Recognizing the many known factors which may enter and alter the results in a study of the protein content of bacteria, we have attempted to control these factors in so far as possible by keeping them constant throughout. In this way, to a certain degree, a comparison is possible between the derived chemical data.

The organisms used in this study were for the most part recovered from sputum, blood, etc., of patients in St. Luke's Hospital, although we are indebted to Dr. Avery, of the Rockefeller Institute, and to Dr. Wadsworth, of the New York State Health Department Laboratories, for a few strains of the Type II. In each instance the identity of the organism was established by its characteristic morphology, staining properties, bile solubility, and reaction to specific immune serum. Ten strains of each of the four chief groups were cultured in flasks containing about 75 c.c. of a beef infusion broth containing one per cent. of dextrose, and having an average reaction of P_H 7.4 to 7.6. Usually sufficient growth for the purpose of the study was produced within 24 hours, when incubated at 36° or 37° C. The sedimented organisms were removed from the bottom of the flask by means of a Pasteur pipette, placed in a Hopkins vaccine tube, centrifuged, and the supernatant fluid removed from above the

packed sedimented bacteria. Physiological salt solution (0.85 per cent.) was added to the sediment in the proportion of 150 to 200 volumes of salt solution to one volume of the packed organism. The organisms were thoroughly washed in this fluid, and then re-centrifuged at a high speed for thirty minutes, until the sediment failed to pack further. The supernatant salt solution was removed, and if necessary, the upper zone of the packed bacterial column in the small calibrated portion of the vaccine tube. Sufficient physiological salt solution was added to make a 1 per cent. suspension by volume. The suspension was subjected to heat in the water bath for one hour at 60° C. to kill the organism. Tests were made for sterility. No preservative was added.

The nitrogen determination was made upon aliquot parts of the killed bacterial suspension.

The total nitrogen content of the bacteria was determined by the micro-chemical method of Folin and Farmer. For this purpose, 1 c.c. samples of the homogeneous suspension of the organism were used for each estimation. The tests were made in duplicate, and if the results did not closely agree in each instance, the tests were repeated. It is noteworthy that the results of such tests showed a close agreement. The total solids were determined by placing 5 c.c. of the homogeneous suspension of the organism in a small tared porcelain crucible, then removing the water content in so far as possible in an electric oven kept at a temperature of 65° to 70° C. Finally the crucibles were placed in desiccator containing phosphorus pentoxide. A vacuum was produced, then it was placed in the electric oven (65° to 70° C.) until the crucible with its contents came to a constant weight. From the total solid content of the dried solids of each specimen, the added sodium chloride (physiological salt solution used in preparation) content was deducted, leaving the amount of total bacterial solids. The percentage of bacterial nitrogen was estimated upon this basis.

In general, the nitrogen contents of the individual strains (ten) in each of the four serological groups of pneumococcus were approximately of the same amount, although an occasional exception was observed, either a slightly higher or a lower value than the general mean, which did not alter the average to any extent. This occurred in the Group I series of determinations

in three instances. New bacterial preparations were made from each strain and the nitrogen contents were determined. The results of the second series showed but slight variation from the first. We are inclined to the view that either a relatively high or a low nitrogen content of certain strains may be an inherent quality, slightly deviating in this respect from the average strain, when grown under parallel conditions. Group III strains seemed to fall into two subgroups when based upon their nitrogen content; one (a) subgroup (four strains) showed an approximately lower percentage of nitrogen than the second (b) subgroup (six strains), which showed a nitrogen content similar to the three other serological groups. A number of possible factors which might cause this variation have been considered, but as yet, no one has proved to be the direct cause. Averages of the results taken from ten strains analyzed in each of the serological groups gave the following total nitrogen content:

Group I, 9.4 per cent.; Group II, 10 per cent.; Group III, 8 per cent. (subgroup "A" gave 7.43 per cent.; subgroup "B" gave 9.3 per cent.); Group IV, 8.8 per cent. As is apparent, the total nitrogen content of the four general serological groups does not greatly deviate from a mean average of 9 per cent., a mean which is reduced by using a general average of 8 per cent. for Group III. It must be emphasized that these values are not considered fixed, since a parallel series might slightly alter the percentage results.

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The transmission of the virus of herpes febrilis along sensory nerves with resulting unilateral lesions in the central nervous system in the rabbit.

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Rabbits inoculated upon the cornea of the right eye with our virus of *Herpes febrilis* show constantly on about the fourth or fifth day a turning of the head toward the right side; during the succeeding days the neck is twisted strongly toward the