gonococci (whose reaction was pH = 6.4) with distilled water. Water, however, was so much less efficient than sodium hydroxide as an extracting agent that its use was abandoned.

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"Nucleoprotein" and Non-Protein Substances Isolated from the Gonococcus. II. Immunological Reactions with Anti-Gonococcus Serum.

ALDEN K. BOOR AND C. PHILLIP MILLER, JR.

From the Department of Medicine, University of Chicago.

Anti-gonococcus sera were prepared by injecting rabbits intravenously with washed, live organisms from 18-hour cultures grown on an agar medium containing a tryptic digest of egg-white.[1] The 5 individual strains employed included recently isolated ones and old stock strains which had undergone some 400 transplantations in this laboratory. The rabbits were obtained from a special stock of snuffle-free animals. Sera against a mixture of stock strains and against the protein fraction described in the preceding paper[2] were also prepared.

No antiserum against the non-protein fraction was obtained.

Precipitin reactions were run with each immune serum using as precipitinogens the protein and non-protein fractions prepared from each of the 5 strains. Ring tests were made by stratifying the precipitinogen, in progressive 10-fold dilution, over the serum (diluted with 2 parts of saline) in small tubes of 4 mm. inside diameter. Readings were made after standing at room temperature for one hour.

The table gives a sample of the results of the precipitin tests on the various protein and non-protein fractions, using rabbit immune sera prepared by the injection of (a) whole organisms of one strain, (b) the "nucleoprotein" of that strain, and (c) whole organisms of a mixture of strains. The titers of the "nucleoproteins" were somewhat higher than those of the non-protein fractions.

It is noteworthy that the anti-nucleoprotein serum gave positive tests with the non-protein substances. This probably indicates the

[1] Miller, C. P., and Castles, R., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 123.

[2] Boor, A. K., and Miller, C. P., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1046.

TABLE I. Titres of Representative Precipitin Reactions.

Precipitinogen	Immune Sera Prepared Against:		
	Whole organisms Strain 1	"Nucleoprotein" Strain 1	Whole organisms Many strains
"Nucleoprotein" strain 1	+++ ++	+++ +	+++ +++
" " 3	+++ +	+++ +	+++ +
" " 5	+++ ++	+++ ++	+++ ++
" " M6B2	+++ ++	+++ +	+++ ++
" " 10	+++ +++	+++ ++	+++ +
Non-protein fraction strain 1	+++	+++	+++ +
" " " 3	+++ +	+++ +	+++ +
" " " 5	+++ ++	+++	+++ +
" " " M6B2	+++ ++	+++	+++ +
" " " 10	+++	+++ +	+++ +

The plus marks (+) indicate the dilutions as multiples of 10; thus: +++ = 1:1,000; +++ + = 1:10,000, etc.

presence in the "nucleoprotein" of the reactive substance of the non-protein fraction, which we believe to be a polysaccharide. In view of the process of purification it may be assumed that the polysaccharide is present as part of the protein aggregate. Another possibility which has not been eliminated is the incomplete removal of the last traces of protein from the non-protein fraction, although it gives no chemical tests for proteins.

No evidence of strain specificity was observed among the 5 strains from which non-protein fractions have been prepared. Casper[3] has reported (in a paper which has but recently come to our attention, although it was published last November) the isolation from gonococci of a non-protein substance, containing polysaccharide, which was type specific for each of 2 types which he was able to differentiate on the basis of agglutination reactions. He made his preparations from bile solutions of gonococci and employed them in a study of the cutaneous reactions of patients suffering from gonorrhoea.

[3] *Klin. Wochsch.*, 1930, **9**, 2154.