changes in the cord) we note that the latter value represents slightly more than one-third of the former. Although anatomically we placed the electrodes at a point about midway in the Achilles reflex arc our mean latency value is much less than half the mean Achilles reflex response latency value. Electrophysiologically considered, the segment of the cord containing the assumed central portion of the Achilles reflex arc is not the half-way point in the arc. Either more structures or structures with higher resistances to the passage of the electrical changes we are studying must lie between this commonly conceded mid-point and the responding muscle tissue than between the sense organs and such a cord center.

The relatively great variations in latencies found in not only different rats but also in the same rat, although not readily understandable, may be partially explained in that from rat to rat and from record to record in the same rat the depth of anaesthesia varied.

In its possible effect upon the dynamic relationships existing between higher and lower neural levels and upon the conducting circuits mediating between these levels this implied irradiation of the reflex influence would appear to be highly significant.

5666

"Nucleoprotein" and Non-Protein Substance Isolated from the Gonococcus. I. Preparation.*

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The preparations described below were made from 12 to 18 hour cultures of gonococci grown on an agar medium[1] containing a tryptic digest of egg-white from which the heat-coagulable proteins had been removed. The organisms were taken up in saline, centrifuged, washed and again centrifuged until well packed. They were then extracted by suspending each cubic centimeter of packed, moist organisms in 200 cc. of N/100 NaOH and allowing the suspension to stand over night in the refrigerator. After removal of the bac-

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

terial debris by centrifugation the clear supernatant liquid was acidified with acetic acid to maximum precipitation.

The precipitate comprising the so-called bacterial "nucleoprotein" was separated from the supernatant liquid (the subsequent treatment of which is described below) by centrifugation, redissolved in N/100 NaOH, and reprecipitated with dilute acetic acid. After 4 to 6 repetitions of this procedure, the final solution of the "nucleoprotein" in N/100 NaOH was dialysed against distilled water and the neutral suspension evaporated to dryness at 40°-60°C. in an air current. In the dry state it has been kept at room temperature for several months without evidence of deterioration. The usual reactions of proteins were all positive: biuret, Millon, Xanthroproteic, Hopkins-Cole, sulpho-salicylic and phosphotungstic acid.

The non-protein fraction was obtained from the supernatant fluid of the first acetic acid precipitation. This liquid was evaporated to small bulk (about 10 cc.) heated in a boiling water bath for 15 minutes, filtered from coagulated protein and then treated with about 10 volumes of 95% ethyl alcohol. After standing long enough to insure complete denaturation of any denaturable proteins the precipitate was removed by centrifugation, dissolved in water, filtered, reduced to small bulk by evaporation and again treated with alcohol. After 2 or 3 such reprecipitations the aqueous solution was allowed to stand over night in the refrigerator and dialyzed against distilled water. The neutral solution was then evaporated to dryness in the same manner as the protein fraction. Like the latter it has been found to keep satisfactorily in the dry state at room temperature.

This non-protein fraction gave a positive Molisch test. The Benedict-Fehling reaction was negative before and positive after acid hydrolysis. All of the tests for protein enumerated above were negative. It appears, therefore, to consist largely of carbohydrate, presumably a polysaccharide.

The unexpected non-specific reactions described in the third paper of this series[2] aroused the suspicion that the repeated treatment with N/100 NaOH might have been so heroic as to have denatured the isolated substances sufficiently to alter their antigenic properties. This possibility was ruled out by checking an adequate number of precipitin reactions with preparations made by extracting washed

[2] Boor, A. K., and Miller, C. P., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1050.

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.

5667

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

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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.