lation between orange pigment, the ability to ferment mannite or produce coagulase on the one hand and susceptibility to lysozyme on the other.

## 8348 P

## Quantitative Use of Neufeld Reaction with Special Reference to Titration of Type II Antipneumococcic Horse Sera.

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(Introduced by A. F. Coca.)

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The Neufeld reaction has recently been advocated[1] for the diagnosis of the different types of pneumonia directly from the patient's sputum. For this purpose, he advised a monovalent antipneumococcic rabbit serum in order to avoid the non-specific reactions which he obtained with the use of antipneumococcic horse serum. More recently, Cooper and Walter[2] reported this same "swelling" phenomenon when antipneumococcic serum from rabbits was mixed with the homologous pure culture grown in artificial media. This observation led us to examine different samples of antipneumococcic serum by mixing them with specific pure cultures and observing the degree of capsular swelling which resulted. It was found that a serum high in mouse protective units caused much more swelling than a serum low in mouse protective units, also that a serum of high potency could be diluted many times and still give a typical "swelling" reaction. These experiments suggested a possible quantitative application of the Neufeld reaction.

Preliminary work with Type II broth cultures and specific antipneumococcus horse sera showed a definite linear relationship between the number of organisms used and the least amount of antibody required to produce an enlarged capsule. This reaction appeared to be specific. The following method of titration of Type II antibody was then developed:

The culture, either live or formalinized, is diluted with 1% peptone to the required density; the serum is diluted with physiological saline solution. Appropriate amounts of a constant culture dilution and of varying serum dilutions are measured with standard platinum loops, placed on a thin cover-slip, mixed well with a loopful of

[1] Sabin, *J. A. M. A.*, 1933, **100**, 1584.

[2] Cooper and Walter, *Am. J. Pub. Health*, 1935, **25**, 469.

methylene blue solution, and inverted on a microscope slide. These preparations are incubated 30 minutes in a moist chamber at 37°C., and then examined under a fluorite oil-immersion lens. The end-point is taken as the highest dilution of serum producing definite capsular swelling. A control serum of known unit value is used as a basis for comparison and for calculation of potencies of the unknown sera.

TABLE I.

Comparative Values Obtained in the Measurement of Type II Antibody in 53 Samples of Antipneumococcic Horse Sera.

| Horse No. | Capsular-Swelling Units | Mouse Units | Horse No. | Capsular-Swelling Units | Mouse Units |
|---|---|---|---|---|---|
| 4432 | 400 | 600 | 6490 | 150 | 200 |
| 4479 | 175 | 225 | 6491 | 300 | 250 |
| 4616 | 200 | 175 | 6494 | 45 | 50 |
| 5373 | 100 | 25 | 6498 | 75 | 50 |
| 6071 | 85 | 100 | 6499 | 50 | 75 |
| 6222 | 75 | 75 | 6515 | 75 | 75 |
| 6225 | 85 | 50 | 6625 | 25 | 35 |
| 6229 | 50 | 35 | 6626 | 75 | 75 |
| 6309 | 100 | 10 | 6627 | 75 | 75 |
| 6313 | 200 | 175 | 6628 | 25 | 15 |
| 6325 | 150 | 75 | 6629 | 25 | 35 |
| 6333 | 100 | 75 | 6630 | 275 | 300 |
| 6335 | 150 | 100 | 6631 | 25 | 35 |
| 6336 | 225 | 300 | 6634 | 35 | 25 |
| 6369 | 200 | 200 | 6635 | 50 | 50 |
| 6441 | 125 | 50 | 6639 | 75 | 50 |
| 6450 | 60 | 75 | 6642 | 65 | 25 |
| 6451 | 65 | 35 | 6649 | 5 | 2 |
| 6454 | 50 | 50 | 6650 | 175 | 35 |
| 6459 | 175 | 100 | 6653 | 125 | 100 |
| 6461 | 50 | 35 | 6654 | 60 | 25 |
| 6463 | 65 | 10 | 6774 | 75 | 15 |
| 6464 | 10 | 5 | 6775 | 50 | 25 |
| 6466 | 75 | 50 | 6776 | 65 | 10 |
| 6478 | 75 | 50 | 6777 | 25 | 10 |
| 6479 | 125 | 150 | 6831 | 50 | 5 |
| 6481 | 110 | 150 | | | |

This technic was applied to 53 samples of Type II antipneumococcic horse sera which were also assayed by the mouse protection test. The 2 sets of unit values thus obtained were correlated according to the Pearson formula. The coefficient of correlation between the values obtained by the use of the Neufeld reaction and by the mouse protection test was found to be 0.89.

Similarly, 20 samples of Type II antipneumococcic horse sera were tested by the method described and by Felton's equivalent-combining method.[3] The coefficient of correlation obtained from these results by the Pearson formula was 0.91.

[3] Paper read before the American Association of Immunologists, New York City, April 17, 1935.

The method reported with slight variations in technic has been used with similar success in the titration of Type I antipneumococcic horse sera. A limited number of tests with concentrated antibody solutions have given promising results. No prozones have been encountered. Preliminary experiments indicate that the test may be applied to other types of antipneumococcic sera.

Since a positive reaction may be demonstrated with a solution containing less than 5 units of antibody per cc., and since the technic described requires only a small amount of serum, possible clinical applications such as the demonstrations of specific antibody in human sera are suggested.

We have been able to demonstrate capsular swelling in mixtures of Type I antimeningococcic serum with homologous organisms. This reaction suggests many possible applications, such as the diagnosis of the type of meningitis directly from the spinal fluid. It also indicates that with appropriate sera, it may be possible to demonstrate capsular-swelling reactions with other kinds of bacteria.

8349 P

## Polarity in Lethal Action of Electric Current.

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Electric currents have been shown to be capable of directing or determining the growth of certain organisms of simple symmetry.[1,2,3] The present is a report of experiments in which the application of direct electric current resulted in partial death of the organism; the effect being localized in a polar fashion. The results given below were obtained with the Bryophyte *Conocephalus*. Lunularia and Marchantia reacted similarly but not as definitely.

The plants were cleaned. Most of the rhizoids and the apex and base were clipped off. Current was applied through an agar bed (usually containing nutrients) upon which the plants were placed in intimate contact. It was found that death occurred in that part of the plant which lay toward the anode. This effect was not due to the creeping of toxic materials from the electrodes as suitable pre-

[1] Barth, L. G., *Physiol. Zool.*, 1934, **7**, 340.

[2] Lund, E. J., *J. Exp. Zool.*, 1924, **39**, 357.

[3] Schechter, Victor, *J. Gen. Physiol.*, 1934, **18**, 1.