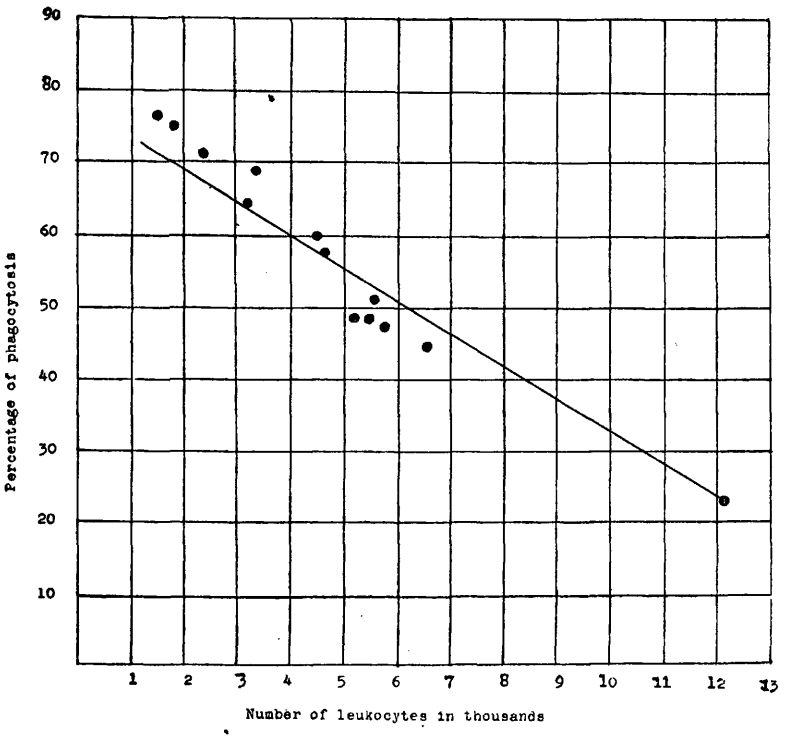

range from 1500 to 12,000 their relation is practically linear, *i. e.*, represented graphically by a straight line with a negative slope. This slope is a measure of the phagocytic power of the individual's leukocytes. (2) The proportionality constant obtained can be used as a basis for comparing values obtained in the study of phagocytic functions of leukocytes of patients undergoing treatment in which the number of leukocytes is changed.

6179

A Purified Protein Antigen for the Complement Fixation Test in Gonorrheal Infection.*

ARCHIBALD MCNEIL. (Introduced by W. H. Park.)

From the Department of Health Research Laboratories, New York City.

The gonococcus antigen used by Schwartz and McNeil in 1910-11 was a simple suspension of 18 to 24 hours old gonococcus cultures

* I want to thank Dr. Annis Thomson and Miss Pauline Bristol for their assistance and hearty cooperation.

of the 10 strains isolated by Torrey, in 0.85% saline solution, preserved with lysol.

This antigen gave strong cross fixation with anti-meningococcus sera, but did not cross fix with the anti-sera of any of the other pathogens tested. It fixed complement with practically all sera taken from known positive cases of gonorrhea and at the time seemed to give very satisfactory results. In 1912, with Olmstead I succeeded in preparing an aqueous extract antigen filtered through a Berkefeld filter that did not bind complement with antimeningococcus sera, and in experimental work was much more satisfactory than the previous antigens used. When injected intravenously into rabbits it was extremely toxic and produced a serum of high titre. But different preparations varied greatly in their antigenic properties, and while its antigenic value was not impaired by temperatures as high as 80°C. for one-half hour, it was unstable when kept for any length of time and tended to become strongly anti-complementary.

In 1916, working with Wilson, the defatted antigen was developed. This antigen was prepared by treating 18 to 24 hour old cultures of gonococci first with alcohol and then with ether, drying and suspending in 0.9% saline solution. This proved to be a very stable antigen that could be heated to 80°C. without lessening its specificity or antigenic properties. This antigen does not cross fix with anti-meningococcus sera and has been used in routine work from 1916 to the present time with satisfactory results when in the hands of experienced and specially trained technicians.

In April, 1931, a research group was formed consisting of Dr. Emily D. Barringer, Dr. Anna W. Williams, and Dr. Archibald McNeil, to make an intensive study of gonorrhea in women, under a special fund given by Lucius N. Littauer. The cases studied were night court cases sent to the Kingston Avenue Hospital as cases of clinical gonorrhea, and kept there until discharged as cured, making ideal material for the study in question.

Dr. Barringer had complete control of the clinical side of the work, and Dr. Williams the bacteriological and cultural side, and Dr. McNeil the serological work.

The combined results of the 3 studies were correlated with very illuminating results.

It was found that while the complement fixation test with the McNeil-Wilson antigen then in use undoubtedly gave positive results in practically all of the cases of gonorrhea during the period of antibody formation, it left considerable to be desired in regard to

doubtful and weakly positive sera. It was found that if the readings of the tests were made in less than 45 minutes after the antigen controls had cleared, positive readings would be obtained with many sera that should give negative readings, and even with this precaution some sera would continue giving weakly positive readings long after the time when the clinical and bacteriological findings seemed to indicate that a cure had been effected. Cases that had given negative results when the sera was freshly drawn would give indefinite results after the serum had remained for several days in the ice box, and was re-inactivated and re-tested. It was found that after treating the gonococci with alcohol and ether and suspending them in 0.9% saline solution they still maintained their typical morphology and Gram staining properties. The suspension precipitated very rapidly and it was often difficult even with constant shaking to maintain a uniform suspension.

In an attempt to remedy this condition, distilled water was added to the dried gonococci powder resulting from the alcohol and ether treatment, and this suspension placed in the waterbath at 55°C. for half an hour, shaking frequently. This treatment destroys the morphology of the organisms but still leaves the cell protein insoluble. The suspension was then centrifuged at high speed for one half hour, the supernatant fluid decanted and the protein residue suspended in 0.9% saline solution. The supernatant fluid was found to have very little antigenic value and contained lytic and anti-complementary substances. The residual protein suspended in 0.9% saline was found to have lost none of its antigenic strength and to be uniform in its action and to give much cleaner cut results than before the distilled water extraction. The appearance of the suspension was much improved, being much more homogeneous and precipitating very slowly on standing.

But it was still not quite satisfactory as an antigen for routine work. Negative results were still delayed with many known negative sera and apparently cured cases continued to give indefinite reactions. I had previously tried treating the fresh gonococcus cultures with benzine and carbon tetrachloride in place of alcohol, but found them both unsatisfactory. I now tried treating the dried gonococcus powder, that had already been extracted with alcohol and ether, with carbon tetrachloride. The carbon tetrachloride was added to a tube containing the gonococcus powder, and the tube shaken thoroughly. It was then placed in the water bath at 55°C. for 5 minutes, then centrifuged at high speed for 5 minutes. When the tubes were removed from the centrifuge it was found that the pro-

tein matter was floating on the tetrachloride at the top of the tubes while in the bottom of the tube was a considerable amount of crystalline matter.*

The tetrachloride together with the protein was carefully decanted into a filter prepared with a hard filter paper, being careful not to disturb the crystalline matter in the bottom of the tube. The tetrachloride was filtered off and the protein collected and dried on the filter paper. After thoroughly drying the protein on the filter paper, it was weighed and found to have lost about 1% from the tetrachloride treatment. It was then suspended in freshly double distilled water in the proportion of 1.0 gm. to 200.00 C. C. M. and placed in the water bath at 55°C. for 30 minutes, shaking frequently. The suspension was then centrifuged for 30 minutes at high speed, the supernatant distilled water decanted, and the protein sediment suspended in 0.9% saline solution, containing one part in ten thousand of mertheolate as a preservative. The amount of saline added was equal to the distilled water discarded. The distilled water portion was found to be only feebly antigenic, while the protein residue gave a suspension unimpaired in antigenic value and has proven to be specific, and give clear cut positive readings with known positive sera. The negative results were clear cut and most of the readings could be made within 10 minutes after the antigen controls had cleared. Negative sera have been reinactivated and retested day after day with uniformly clear cut negative results and many questionable and indefinite plus-minus results given by the old antigen have been eliminated.

We are now using 5 Torrey strains of gonococci (2 of which are master strains) in the preparation of antigens.

Preparation of antigen for routine work. Five gms. of dried gonococcus powder of each of the 5 Torrey strains, prepared as previously described, is suspended in 1000.00 C. C. M. of freshly double distilled water, this suspension is placed in the water bath at 55°C. for 30 minutes, shaking frequently. The suspension is then centrifuged at high speed for 30 minutes, the supernatant fluid discarded and the remaining protein residue suspended in 1000.00 C. C. M. of 0.9% saline containing one part in ten thousand of mertheolate. This antigen can as a rule be used in a one in 20 dilution in routine work and seems to be free from the undesirable properties of the antigens previously used. One hundred sera of clinical cases have been tested with most satisfactory results. With the

† The amount of crystalline matter varies greatly in different cultures grown on different lots of medium.

antigen as now prepared it should be possible for any technician, who can properly standardize complement and perform satisfactory Wassermann tests, to obtain accurate and reliable results that will satisfy clinicians, with the complement fixation test of gonorrheal infection.

6180

A "Lipoid" Extract of Spleen that Prevents Bartonella Muris Anemia in Splenectomized Albino Rats.

DAVID PERLA AND J. MARMORSTON-GOTTESMAN.

From the Laboratory Division, Montefiore Hospital, New York.

The authors[1] have demonstrated that minute splenic autoplastic transplants made 7 weeks prior to splenectomy protect a large percentage of splenectomized rats from *Bartonella muris* anemia. A comparative histological study of the transplants of protected and unprotected rats revealed a regeneration of the pulp cells in the protected rats and an exhaustion destruction of the pulp in the unprotected rats. This supported the hypothesis that the reticular and endothelial cells of the pulp of the spleen possess some internal secretory substance. The parabiotic experiments of Lauda[2] further support the internal secretory action of the spleen. He found that rats joined by parabiosis are protected if the spleen of only one animal is removed.

Many investigators have tried and failed to demonstrate some substance in the spleen which would replace the spleen in protecting adult splenectomized rats against *Bartonella muris* anemia. We have made many attempts during the past three years to obtain such an extract. Lipoid extracts of the spleen were prepared which possess the property of protecting splenectomized adult albino rats against *Bartonella muris* anemia in a large percentage of instances. Since the anemia in the male is more severe, only male rats were used in testing these extracts. Of 440 male rats of carrier stock used for studies of *Bartonella muris* anemia during the past 3 years, not a single rat failed to develop *Bartonella muris* anemia following splenectomy.

[1] Perla, D., and Marmorston-Gottesman, J., *J. Exp. Med.*, 1930, **52**, 130.

[2] Lauda, E., and Flaum, E., *Z. ges. exp. Med.*, 1930, **73**, 293.