PROCEEDINGS OF LOCAL BRANCHES OF THE
SOCIETY OF AMERICAN BACTERIOLOGISTS

WINTER MEETING OF THE EASTERN MISSOURI BRANCH

School of Medicine, Washington University, St. Louis, Mo.,
February 7, 1939.

**Epidemic of Nursery Diarrhea.**

J. P. Costello, St. Louis County Hospital, St. John’s Hospital; H. E. Lind, St. Louis County Health Dept., St. Louis County Hospital.

This report includes a series of 26 infantile diarrhea cases with 11 deaths occurring at the St. Louis County Hospital during the Winter—Spring season of 1938.

From a bacteriological investigation we feel that in this epidemic we were dealing with a pathogenic strain of *Proteus vulgaris* associated with *Escherichia coli*. We feel too that a contributing factor is the absence, or an extremely low content, of free hydrochloric acid which probably favors the growth and production of such bacteria capable of producing a toxic condition as found in these infants. We realize of course, the possibility that this organism *P. vulgaris* and its possible toxins may not have been the primary cause of these diarrheas but may have been secondary.

We feel that our epidemic was similar in many respects to those reported in various hospitals throughout the temperate zone. We feel, too, that the severe toxemia was a result of the absorption of toxins from bacterial development in the gastro-intestinal tract.

We fully realize that *Proteus vulgaris* is found in healthy individuals, but is rarely found predominantly in the intestinal tract and rarely if at all in the stomach, except under pathological conditions.

The disease was characterized by its constantly severe and frequently fatal toxemia. Its onset, cause and duration is so similar in these cases as to strongly suggest that this disease be classified as a communicable disease.

**Reinfection in Poliomyelitis.**

Edwin H. Lennette, and Francis B. Gordon, Department of Pathology, Washington University School of Medicine, and the Department of Bacteriology and Parasitology, University of Chicago.

Eighteen rhesus monkeys recovered from infection with the MV virus strain were inoculated nasally with the same strain; only two of these responded, showing weakness and fever. In the light of the criteria laid down by Sabin and Olitsky, these monkeys represent, at best, questionable cases of reinfection.

Neutralization tests prior to administration of the heterologous virus 655 on September 23, 2017 by guest
revealed that antibodies were present against the MV but not against the Philadelphia strain. After infection with the Philadelphia virus, antibodies against this strain appeared in the serum. Immunologic differences between the two strains are described.

THE AGGLUTININ RESPONSE TO BRUCELLERGEN. T. E. Kircher, Jr., Department of Medicine, Washington University, St. Louis, Mo.

Skin tests with Huddleston's Brucellogen were performed on a series of patients who previously had shown no serum agglutinations against Brucella abortus strain number 456. Fifty of those having negative skin reactions; i.e. no edema, induration or erythema persistent at 72 hours, were retested for the presence of agglutinins after intervals of from one to eight weeks. Forty of these, or 80 per cent, had a definite response; i.e. showed an agglutination of B. abortus No. 456 in titers of 1-40 to 1-640 dilution. There was no similar response to skin tests by either rabbits or guinea pigs.

It is suggested, therefore, that in cases of suspected Brucellosis, agglutination tests should be performed preliminary to skin testing with Brucellogen lest subsequent tests reveal agglutinins to a titer so high that a false diagnosis is encouraged.

CHICK EMBRYO INOCULATION AS A DIAGNOSTIC AID IN VARIOLA AND VARICELLA. Floyd S. Markham and George S. Bousis, Department of Bacteriology, School of Medicine, Washington University, St. Louis, Mo.

During a small outbreak of variola in the winter of 1938-39, atypical cases were frequently observed which were difficult to differentiate from varicella. The technique of chick-embryo inoculation was employed as a diagnostic aid. Strains of variola virus were isolated by direct inoculation of vesicle content onto the chorioallantoic membrane in 5 instances associated with atypical symptoms or lesions. Variola virus was identified by neutralization test performed on the chick membrane with anti-viariola or anti-vaccinia rabbit serum.

Vesicle fluids from 5 cases of varicella and 4 of herpes zoster failed to produce infection of the chick membrane.

NOTES ON THE KAHN REACTION WITH ANIMAL SERA. Wm. H. Gabby, City Hospital Laboratory, St. Louis, Missouri.

INCLUSION BLENNORRHEA. L. A. Julianelle, School of Medicine, Washington University, St. Louis, Missouri.

MOTION PICTURE FILM ON RABIES. J. J. Bronfenbrenner, School of Medicine, Washington University, St. Louis, Missouri.

SPRING MEETING OF THE EASTERN MISSOURI BRANCH

School of Medicine, Washington University, St. Louis, Mo., April 11, 1939.

BACTERIOLOGICAL DIAGNOSIS OF GONORRHEA IN THE MALE. Axel Gronau, School of Medicine, Washington University, St. Louis, Mo.

A total of 819 bacteriological examinations was made on 221 male patients, of whom 160 were definitely proven to have gonorrhea by bacteriological ex-
aminations as well as by clinical symptoms. Sixty-one patients were assumed to have some genito-urinary infection other than gonorrhea, and while the actual cause of the infection was not established, at no time were gonococci found by the smear or by the cultural method. Each bacteriological examination consisted of smear and culture, and the efficiency of the smear method and the cultural method as a means of diagnosis was compared.

The smears were stained according to Hucker’s modified Gram’s staining procedure. The culture medium consisted of a 5 per cent rabbit blood chocolate agar. Cultures were incubated at 36°C. for about 40 hours under 10 per cent CO₂ tension. The oxidase technique was employed for the detection of gonococci on the culture.

In 88.16 per cent the findings of smear and culture agreed, and disagreed in 11.84 per cent, taking the 819 examinations as 100 per cent.

The number of total positive findings, where the presence of gonococci was revealed either by smear or culture, or both methods, was 185. Taking this number as 100 per cent efficiency in detecting gonococci, it was found that in 13.52 per cent the culture was able to detect gonococci where the smear definitely failed to do so. In addition in 7.03 per cent the culture revealed gonococci, where the smear was doubtful (±). In 2.70 per cent the smear was positive and the simultaneous culture negative.

An analysis is given of those instances, where the smear was doubtful (±) and the simultaneous culture negative. It was felt that here the deciding factor should be the culture, in the sense, that if no gonococci were found on the culture, the suspected organisms in the smear should be assumed not to be gonococci.

**Observations on Chapman’s System for Determination of Staphylococcus Pathogenicity.** MacDonald Fulton, R. O. Meuther and Sister Agnes Gerard Knowles, St. Louis University School of Medicine and Firmin Desloge Hospital, St. Louis, Mo.

68 of 102 freshly isolated strains of staphylococci were "probably pathogenic" according to Chapman’s PHCVBM system of tests. In addition, 16 strains were coagulase positive but negative in one or more of the tests on crystal violet, brom-thymol-blue or mannitol agars. 18 strains which were coagulase-negative were negative in most other tests.

Results on crystal violet and brom-thymol-blue media were not always reproducible. Among the important factors were the age and amount of the inoculum. Reading of brom-thymol-blue agar tests was complicated by strains giving a greenish growth, instead of the usual orange. The hemolysis test would be improved by some agreement as to what constitutes true hemolysis, as distinct from pseudo hemolysis.

Statistically, there is a significant association between ability to liquefy gelatin and ability to coagulate plasma. The gelatin stab culture method is slow and unreliable. A gelatin agar plate tested with ammonium sulfate proved satisfactory.

Statistical examination of some of the available data (Chi square, r be Sheppard’s method) indicates a high degree of significance for Chapman’s system of assessing pathogenicity when compared to clinical and in vivo findings.

**Notes on the Coagulase Reaction of Staphylococci.** Francis W. DiRocco and MacDonald Fulton, De-
partment of Bacteriology, St. Louis University School of Medicine, St. Louis, Mo.

A study was made of the effect of various factors on the coagulase reaction. Cultures and plasma (rabbit) could be aged several weeks without altering the result of the test. Eight washings of suspensions from agar slants failed to remove or appreciably decrease the coagulase activity of the cells. Coagulating activity was observed in the washings, suggesting that coagulase, although closely associated with the cells, is an extracellular product. Coagulation was more rapid in cultures grown in broth decalcified with oxalate than in controls. Positive reactions were obtained more rapidly when tests were conducted in shallow layers. At room temperature twice as much time was required to form clots as at 37°C. Room-temperature tests were difficult to read, the clots being threadlike in appearance. Production of coagulase was considerably decreased when organisms were grown in an atmosphere of about 25 per cent CO₂.

ANISEPTIC VALUE OF CERTAIN PHENOLIC OINTMENTS. W. C. Clark, James F. Ballard, Inc., St. Louis, Mo.

The author prepared a series of 2% Phenol Ointments in several fatty bases, he also added certain agents he thought might act as partition agents to carry the phenol away from the fat. Petrolatum; Petrolatum with added fat-like bodies such as; Castor Oil, Cocoaenut Oil, Hydrogenated Castor Oil, "Crisco," Soap, Sodium Lauryl-sulfonate, Glycerin, Water, Woolfat, Proteginx, Glyceryl Monostearate and Propylene Monostearate were each made into Phenol-petrolatum Ointments. When these Ointments were tested on 10% serum agar plates using F.D.A. method with Staphylococcus aureus A 209 as test organism, not one produced a zone of inhibition!

Phenol, Thymol, Chlorothymol, Para-chlor-metaxylenol, Para-amylyphenol, Para-phenyl-phenol, and Santopenh "7", each were made into Ointments from each of the following fatty bases; Petrolatum, Crisco, Spry, Hydrous and Anhydrous Woolfat, and Vanishing Cream. The phenolic strength was 2% except in the case of Phenol which was boosted to 5%.

Tests indicated that Petrolatum was probably the best fatty base for this group, because the best zones obtained were in the Petrolatum Ointments of (1) Parachlor-metaxylenol, (2) Santopenh "7", and (3) Para-amylyphenol. Vanishing Cream at first appeared to be an ideal base, but prolonged exposure to warmth caused a gradual recession of the zone until the size was less than that of the Petrolatum Ointment.

It was observed that the addition of any one of the following agents usually increased width of the zone: Alcohol 2%, Sodium Lauryl-sulfonate 2%, Glycerin 2%, Water 2%, Soap 2%, Glycerol 2%, Water 2%. Of all the Ointments tested only those made from Parachlor-metaxylenol, Santopenh "7" and Para-amylyphenol had decided zones when tested on 10% serum agar plates using F.D.A. technique and S. aureus for test organism.

The author believes the Phenolic substances like perfumes, are held by the fatty molecule by a sort of absorption which prevents the diffusion from taking place by the ratio of solubilities as it normally should.

THE EFFECT OF 5% SODIUM CHLORIDE ON VARIOUS ORGANISMS; THE EFFECT OF BACTERIOPHAGE ON KREUGERS STAPHYLOCOCCUS AT THIS SALT CON-
THE EFFECT OF 5-PER-CENT SODIUM CHLORIDE ON VARIOUS ORGANISMS. Helen Norris Moore, Fouke Fur Co., St. Louis, Mo.

During a course of studies of obligate Halophilic organisms, it was noticed that the growths were always smooth and sometimes quite stringy. Since a smooth variant was always encountered in growths of these organisms in high salt media, it was decided to endeavor to cultivate ordinary bacteria under the same conditions and observe the effect of high osmotic pressures.

A medium containing not less than 5% Sodium Chloride, 5% Gelatin, eggs, soluble starch, beef extract and agar was finally established as the one having the highest content of salt and still able to support good visible growth. The first transplant to the salt medium from ordinary medium will grow in 48 hours and after that 24 is sufficient. The cultures used, together with the number of transplants on the salt medium were as follows:

<table>
<thead>
<tr>
<th>No. of Transplants</th>
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<tbody>
<tr>
<td>3 Rough Eberthella typhosa (Shwartzman*)</td>
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</tbody>
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* These cultures were supplied by Dr. G. Shwartzman. Their characteristics are described in J. Exp. Med. 1930, 52, 781-903.

All stock cultures of E. typhosa on hand at Washington University.................. 12
All stock cultures Escherichia coli on hand at Washington University.................. 12
Smooth E. coli (fresh isolations).................................................. 101
Proteus X-19................................. 76
Shigella dysenteriae (Shiga)............................. 25
Staphylococcus aureus 209............. 49

The following observations were made:
1. The medium must contain eggs and not less than 5% gelatin in order to obtain good visible growth.
2. The colony appearance of all these organisms on the salt medium is of the normal smooth type. The Shwartzman strains appeared smooth as long as they were cultivated upon the salt medium but reverted at once to their typical morphology as soon as they were transplanted to media of ordinary salt concentrations.
3. The gram-negative bacteria show variations in shape and staining when grown on this salt medium. Elongated, branched and curved cells are common, while in the gram stain the gentian violet is difficult to remove. The cells, however, resume their normal shape and stain when cultivated on ordinary medium.

THE EFFECT OF BACTERIOPHAGE ON STAPHYLOCOCCUS K IN A 5-PER-CENT SALT MEDIUM. Jack Mehl Burnett; Department of Bacteriology, Washington University School of Medicine.

Having learned (personal communication) that Moore was able to maintain a variety of organisms on a special medium containing 5 per cent salt without ever observing the appearance of rough variants, we were
interested to see what effect the presence of salt might have on the susceptibility of these cultures to bacteriophage.

It was found that Staphylococcus K would grow slowly in a liquid medium consisting of 5 per cent salt, 5 per cent gelatin, eggs, soluble starch, and meat extract, and would remain susceptible to its homologous phage for at least 8 hours. Despite the fact that this organism rapidly becomes rough on this medium after 8 hours, it should not be considered as contradictory to the observations of Moore for the organism very frequently becomes rough on ordinary meat-extract media.

This medium by itself has no deleterious effect on phage, so that the titre of phage which has been incorporated in this medium is not altered in any manner.

If this medium is inoculated with approximately $1 \times 10^{7}$ organisms per ml. along with about $5 \times 10^{4}$ units of homologous phage per ml., and incubated at $37^\circ C.$, there is observed a marked growth stimulation in comparison with that obtained in the same medium without the presence of phage. This stimulation was first evident at the end of the first hour, reached its maximum in about 2½ hours, and disappeared with the beginning of lysis after some 4½ hours. The maximum stimulation observed was about 100 per cent over the phage-free control. There apparently is no active phage production during the first two hours and then the phage titre increases about tenfold by the sixth hour. All bacterial counts were made on 2 per cent nutrient agar plates of normal salt concentration, and phage titrations were carried out by the dilution method, using five tubes of each dilution.

This stimulatory effect was not seen when 5-per-cent gelatin was added to nutrient broth of normal salt concentration and the same experiment was carried out.