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Biological Properties of "Fresh" and "Stock" Strains of the Meningococcus.

GEOFFREY RAKE. (Introduced by L. T. Webster.)

From the Laboratories of The Rockefeller Institute for Medical Research, New York.

A study has been made of the biological differences in freshly isolated and old stock strains of the meningococcus. The majority of freshly isolated strains present colonies on blood agar which are pearly in color, lenticular and flattened in form, round in outline, and distinctly moist. While single colonies measure up to 4 mm. in diameter after 24 hours' growth, there is a tendency to coalesce with the formation of a sheet-like growth. By the end of 36 hours the original growth tends to become transparent and glassy, sometimes with the formation of minute, glistening crystals on the surface; new and opaque, yellowish white growth appears at the margin usually in the form of papillæ, and this tends to grow over and obliterate the original glassy colony. While, save rarely, transplantation from the original growth is unsuccessful, subculture can be made from the marginal growth during the next 48 or 72 hours. The colonies so obtained are usually more opaque and domed than in the original culture. Individual colonies are mostly smaller and show less tendency to coalesce, but there are some larger, flattened colonies resembling those of the original culture, giving the plate a characteristically uneven growth. Further subculture accentuates those characteristics noted for the secondary growth—the colonies become smaller and more domed with less tendency to coalesce. Most of the older strains show opaque white colonies but in occasional stock strains the colonies are transparent. They remain viable on blood agar for 48 or 72 hours. In many the formation of crystals becomes well-marked at the end of 48 hours and subcultures are no longer successful. Rough forms have appeared in only one strain,—a stock Type IV culture with considerable yellowish pigment formation. These colonies present a definitely wrinkled surface and emulsify with difficulty in saline; they resemble the colonies of *Micrococcus pharyngeus siccus* but tend to revert readily to the smooth form. Freshly isolated strains show no hemolysis after 24 hours' growth, though there may be some at the end of 48 hours. Older strains often show quite marked hemolysis within 24 hours.

Many investigators have suspected the presence of a capsule in

the meningococcus but Baker[1] alone has shown its existence with stained preparations. By using a modified Baker capsule stain, well-marked encapsulation can be demonstrated in freshly isolated strains; in older strains this capsule becomes progressively less and the rough form shows no capsule.

The acid agglutination range of the meningococcus fails to show the clear cut picture found with many other organisms. Fresh strains, however, do differ from older and more especially from the stock strains. Freshly isolated strains show a relatively narrow zone, ranging from about pH 3 to pH 5 with occasional strains going down to pH 2.7 and a very few up to pH 5.6. After several transplants the zone becomes broader and moves to the acid side, most cultures agglutinating between pH 2.1 or pH 2.3 and pH 4.7, while some go out to pH 5. The stock strains show marked irregularity. The zone is still broader and the majority of strains agglutinate from pH 2.1 or pH 2.3 out to pH 5, pH 5.6, or even pH 6.3.

The range of acid agglutination explains the occasional appearance of salt sensitivity amongst fresh cultures and its more frequent occurrence in the stock strains. Owing to the absorption of CO_2 from the air, saline solution prepared from ordinary distilled water gives a pH between 5.5 and 6. This is within the range of agglutination of occasional fresh strains and several stock strains.

Both protein and carbohydrate fractions can be obtained from a solution of lysed meningococci.* The carbohydrate portion can be separated into 2 parts. One, the more abundant of the 2, is precipitated by about 8 volumes of 95% alcohol. It is readily redissolved in distilled water and can be freed from non-carbohydrate substances by repeated precipitation. It is neither type nor species specific, giving a precipitate with antimeningococcal, antigonococcal and antipneumococcal (especially Type III) sera. It does not precipitate with antistreptococcal sera or with sera prepared against the typhoid and dysentery organisms.

The second carbohydrate fraction has been obtained from fresh strains in smaller amounts. It is precipitated from solution by 1½ to 2 volumes of 95% alcohol and is readily soluble in distilled water so that it can be purified by repeated precipitation. It is type specific, giving a precipitate with some samples of antimeningococcal polyvalent sera, while the fractions obtained from each of the 4

[1] Baker, S. L., *Brit. J. Exp. Path.*, 1920, **1**, 127.

* Zozaya has recently reported a non-specific polysaccharide for this organism (*J. Exp. Med.*, 1931, **54**, 725) and Przesmycki (*J. Inf. Dis.*, 1924, **35**, 537) has briefly described the isolation of a type specific carbohydrate.

types show, in high dilutions, precipitation by the homologous type serum. This fraction corresponds therefore to the S or soluble specific substance of other organisms.

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Immunological Differences Between a Strain of Monkey Virus and Human Poliomyelitis Virus.*

ELLIOTT R. WEYER. (Introduced by William H. Park.)

From the Department of Bacteriology of the New York University and Bellevue Hospital Medical College.

Having succeeded in producing potent antiserums to monkey poliomyelitis virus by the injection of horses with increasing doses of a suspension of infected spinal cords, the occasion of the recent epidemic of polio in New York City has made it possible to determine the potency of these antiserums as neutralizing substances for freshly isolated strains of virus. It has been shown[1] that concentrates from these antiserums were of very high titre and were effective in protecting monkeys already infected with poliomyelitis virus of monkeys as well as bringing about virus neutralizations *(in vitro)* in very high dilution. In this respect these serums were approximately 5 times as potent as human convalescent serum. With continued injection of horses and continued passage of the virus through monkeys a serum has been evolved which neutralizes monkey virus in a dilution upward of 1:500 in spite of the fact that the virus is now more infective for monkeys than it was previously (5% virus, M.L.D. 0.05 cc.) while human convalescent serum neutralizes

TABLE I.
Amount of Antiserum Required to Neutralize.

	Human Convalescent Serum	Horse Concentrate
Monkey Virus No. 1 in 1930, 5% emulsion	20:1	100:1
Monkey Virus No. 1 in 1931, 1% emulsion	5:1	200:1
Monkey Virus (Nasal) No. 2 in 1931, 5% emulsion	20:1	500:1
Human Virus 1931 epidemic, 5% emulsion	50:1	20:1

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[1] *J. Exp. Med.*, 1931, **53**, 553.