organisms quickly disappeared from the blood stream; whereas in rabbits receiving soluble substance and similar amounts of pneumococcus culture, an increase in the bacteremia ensued and frequently terminated in the death of the animals. In the light of Tillett's[8] work, the course of bacteremia in these 2 series of animals may be interpreted to mean that the pneumococcus soluble specific substance causes an increased virulence of the organism. On the other hand, the injection of a culture of a "Rough" variant derived from Pneumococcus Type II after the animals had received similar amounts of the soluble substance failed to produce similar effects.

Experiments with the injection of Type II pneumococcus culture broth instead of the purified soluble substance gave practically identical results as the latter.

Further study on the effect of the injection of Type II pneumococcus soluble substance on the mortality rate of the rabbits which were subsequently infected with the homologous organism, suggested an increase in the susceptibility of the animals or an increased virulence of the organism by 10,000-100,000 times. Similar experiments in which the infecting organism was substituted by a "Rough" variant of a Type II pneumococcus again failed to produce such action. None of these latter animals even seemed to have suffered in any way by such injections.

6082

Use of Antipneumococcus-Serum-Agar for the Identification of Pneumococcal Types.*

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It is generally recognized that Type III pneumococci grow on blood agar plates in large mucoid transparent colonies. This peculiar growth characteristic of the organism has frequently rendered its isolation from plates quite simple. The identification of the other types of pneumococci by the appearance of their colonies, on the other hand, is beset with great difficulties, if not an impossibility. Such identification usually requires agglutination tests carried out with cultures obtained from the individual colonies.

[8] Tillett, W. S., *J. Exp. Med.*, 1927, **45**, 1093.

* We are indebted to Dr. A. B. Wadsworth for the antipneumococcus horse serum employed in this investigation.

In a further study on the *in vitro* transformation of pneumococcal types, where at times the presence or absence of 2 different types of pneumococci, *e. g.,* Type I and Type II, is to be determined, numerous colonies would have to be picked and typed with known immune sera. It was felt, therefore, that a simpler method of identification would save considerable amount of time and material, and render such a study easier technically.

Virulent pneumococci elaborate, during their growth, a soluble specific substance which gives specific precipitation with homologous immune serum. Therefore, it was thought reasonable that if type specific antipneumococcus serum be added to the agar medium, any homologous soluble specific substance elaborated around each colony of pneumococcus might give these colonies a different appearance.

Plates were accordingly made with plain beef infusion agar pH 7.8, to which 1% dextrose and varying amounts of Type I antipneumococcus serum were added, and a culture of Type I pneu-

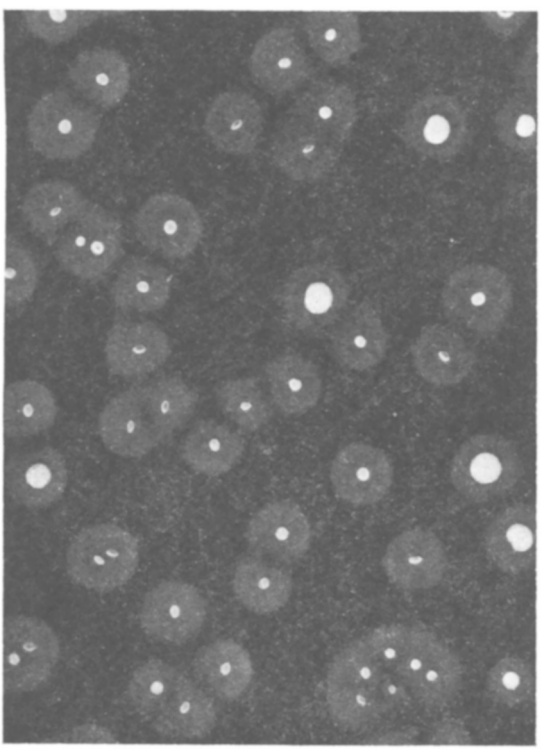

FIG. 1.

Type I pneumococcus colonies grown in Type I antipneumococcus-serum-agar. Showing annular opacities surrounding each colony. × 3.

mococcus was used to streak the plates. However, the results were entirely disappointing. The colonies on all the plates revealed no distinguishing features differentiating them from those grown on plain agar plates.

Poured plates were next employed, and this procedure gave the desired results. With Type I, II or III antipneumococcus serum added to the agar, the homologous organism grown in the depths of the medium after incubation showed annular opacities with a very definite outer margin, surrounding each colony. This was best seen by viewing the plate with illumination from the side on a dark background, and was usually large enough so that it could be seen with the naked eye (see Fig. 1). Colonies that had grown at the bottom or at the surface of the agar tended to spread out, forming large opaque colonies which may be mistaken for the annular opacity just described; therefore, due precaution should be taken when examining these plates. The formation of the annular opacity around the colonies has been found to be strictly type specific.

The amount of antipneumococcus serum to be employed is of great importance. The higher the precipitin titer of an immune serum, the smaller the amount of serum is to be added to the agar. Results similar to the so-called inhibition zone[1] have been observed, in which smaller amounts of immune serum yielded good results while larger amounts negative or poor results. With the diagnostic antipneumococcus sera obtained through the courtesy of Dr. A. B. Wadsworth of the New York State Department of Health, the optimum amounts in the medium have been found to be as follows: For Type I, 3-4%; Type II. 5-6%; and Type III, 1-2%. It is recommended, however, that the optimum amount for each lot of immune serum be tested prior to the use of the immune serum-agar medium.

The length of incubation of the inoculated plates is also of importance. Twenty-hour incubation has been found to be highly satisfactory. Shorter periods of incubation show the annular opacity poorly, while with longer incubation, these annular opacities became larger and finally coalesced, thereby giving rise to a homogeneous clouding of the agar, and defeated the purpose of the medium.

The method now adopted is as follows: 0.3 cc. antipneumococcus serum Type I (or the corresponding optimum amount of immune serum of the other types) is placed in a Petri dish (100 x 10 mm.). 0.5 cc. 20% dextrose solution is also placed in the same Petri dish

[1] Morgan, H. J., *J. Immunol.*, 1923, **8**, 449.

but not allowed to be mixed with the immune serum. A small loopful of a 1:100 dilution of an 18-hour pneumococcus culture is then inoculated into the plate by washing it in the dextrose solution. 10 cc. molten beef infusion agar pH 7.8 cooled to 40-42°C. is then poured and the contents of the plate mixed thoroughly. The plate is then incubated for 20 hours at body temperature, and then examined. Colonies surrounded by annular opacity as described above indicate the same type of pneumococcus as that of the immune serum used.

Working with such a method, mixed cultures of Type I and Type II pneumococci have been rapidly identified and again grown in pure cultures. These cultures have been found to retain their original characteristics.

Summary. A medium consisting of 1% dextrose beef infusion agar pH 7.8 and type specific antipneumococcus serum is presented. Pneumococci of the homologous type when grown in the depths of the immune serum-agar plate develop well defined annular opacity surrounding each colony. The development of the annular opacities has been found to be strictly type specific.

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Action of Ephedrine on Peripheral Blood Vessels as Observed in "Transparent Chamber Preparations".*

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Six rabbits with transparent chambers introduced into the ears according to the Sandison[1] technique were studied. The blood vessels studied included both preformed, and newly formed ones of from 10 days to 4 months old. The reaction to ephedrine of these various types of vessels was essentially the same.

Ephedrine hydrochloride in 0.1% to 1.0% solutions in 0.9% saline was introduced by intravenous injection into the marginal vein of the unobserved ear or the saphenous vein of the leg and continuous observation with the microscope under low power was made during the first half hour after the injection.

* This study was started at the Marine Biological Laboratory at Woods Hole, Mass., and a preliminary note was communicated to Dr. K. K. Chen[2] in 1928, to whom the writer is indebted for one of the samples of ephedrine hydrochloride used.

[1] Sandison, J. C., *Am. J. Anat.*, 1928, **41**, 447.