

was the only symptom seen, although in the 3 that died the terminal behavior was not witnessed. All rabbits displayed at autopsy extensive extravasations of blood in various internal organs.

The animal with 9 cc. per kg. exhibited marked tendency to bleed from cutaneous abrasions, became weak, and had hemoptysis just before death a few hours after the third injection. Organs and tissues showed extensive hemorrhages.

It is concluded that "Thorotrast", probably due to its content of thorium, given intravenously to rabbits tends to lower the thrombocytic content of the blood and to produce acute purpura hemorrhagica. Amounts 3 or more times the standard dose are usually fatal. The fact that the standard dose was given in 2, rather than in 4 or more fractions as given by Kadrnka, may well account for the difference between our results and his.

5822

Use of Dog Blood Agar for the Differentiation of "S" and "R" Pneumococci.

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In the course of a study on the dissociation of pneumococcus, it became evident that the present method of studying the colony morphology by the use of normal rabbit or horse blood agar plates is not highly satisfactory. This is particularly true in the case of "R" strains derived from Type II pneumococci. When grown on these media the colonies of these "R" strains frequently appear so smooth as to make them almost indistinguishable from their original "S" strains. While many of the "R" pneumococci can be recognized by such a method, yet careful observation and a certain amount of experience and familiarity with the technique, as regarding the degree of light, reflection and magnification are necessary. This report deals with the results obtained by the use of the same medium but slightly modified, the employment of which renders the above qualifications unessential.

It has been found that when making up the blood agar plates (the agar employed has been the ordinary beef infusion agar pH 7.8), if substitution is made of the normal rabbit or horse defibrinated blood, by that of the normal dog, the growth of "S" and "R"

pneumococci on such a medium appears so different in the morphology of their colonies that the differentiation between them becomes extremely simple. With a moderate degree of magnification the "S" colonies are seen to be smooth and shiny, while the "R" colonies, including those derived from Type II "S" pneumococci, reveal a wrinkled and coarsely rough surface (see Plate I). It has also

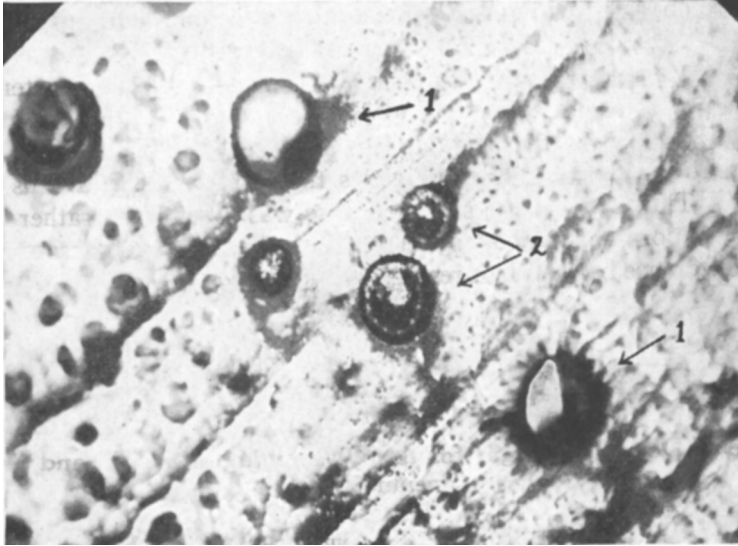


FIG. 1.

1, "Smooth" *Pneumococcus* colonies. 2, "Rough" *Pneumococcus* colonies. Mixture of *Pneumococcus* Type II ("S") and its variant ("R") grown on dog blood agar. $\times 28$.

been observed that pneumococci grown on this medium show marked hemolytic properties. Each colony is surrounded by a wide zone of hemolysis as is seen with hemolytic streptococci.

Defibrinated blood from other normal laboratory animals, such as the guinea pig, white rat, chicken and cat, has also been tried in place of the dog's blood, but none has been found to exert a similar effect on the growth of the "R" pneumococci as that of the dog's blood.

That this property of the dog's blood resides in the cellular elements rather than the plasma is seen in an experiment where dog plasma and washed dog blood cells were mixed with equal parts of horse washed blood cells and horse plasma respectively, and the resulting mixtures employed in making plates. Table I gives the results of growth of "S" and "R" pneumococci on these plates. It is seen that the dog cells are the essential elements which impart to

TABLE I.

Agar plates made with		Differential quality of media for growth of "S" and "R" pneumococci.
Type of plasma	Type of washed blood cells	
Dog	Horse	0
Horse	Dog	+
Dog		0
	Dog	+
Controls		
Dog	Dog	+
Horse	Horse	0

+ Satisfactory. 0 Not satisfactory.

the medium this highly differential character. It is to be noted that even when washed dog blood cells were alone employed in making the blood agar medium, the desired results were equally satisfactory. Similar experiments conducted with rabbit plasma and cells instead of those obtained from the horse, yielded identical results. It has also been observed that the dog cells become hemolyzed rapidly by keeping these dog blood agar plates in the refrigerator for a day or 2, and yet the medium retains its differential qualities. This would point to the fact that intact cells of the dog are not essential, and would seem to indicate that the peculiar quality of the medium depends upon the presence of the hemoglobin of the dog.

The optimum amount of dog's blood in the medium was then investigated. Plates made with varying amounts of defibrinated blood of the dog, constituting 5, 10, 15, and 20% of the blood in the agar, were streaked with "S" and "R" pneumococci and incubated for 16 to 18 hours. It was found that 5% of dog's blood in the medium gives the best result, although the amount of blood as high as 10% is almost as satisfactory; and that when the amount of dog blood is in excess of these quantities, while the "R" pneumococci grow out typically rough, the "S" strains show a tendency to early autolysis as evidenced by wrinkling and flattening of the surface of the colonies. This is particularly true with the "S" forms of *Pneumococcus* Type III.

The length of incubation of the inoculated plates is also of importance. Incubation at 37°C. for 16 to 18 hours appears to be the optimum for bringing out the differential character of such a medium.

Working with a medium such as described, mixtures of "S" and "R" pneumococci either immediately mixed or grown together before plating, could be readily differentiated and pure cultures of either "S" or "R" forms again obtained. Dissociation of pneumococci ("S"→"R" change) by growth in homologous immune se-

rum has been observed to occur as early as the second transfer. The "R" colonies stand out in marked contrast to the "S" forms and can be easily detected. Tests carried out with the "S" cultures grown on the dog blood agar medium have shown that they fully retain their original biological characteristics such as in regard to specific agglutination* and high virulence, while "R" colonies invariably give the properties of those of "Rough" cultures.

Summary. A highly satisfactory and differential medium for the study of "S" and "R" forms of pneumococci is described. The medium consists of ordinary beef infusion agar pH 7.8 to which 5 to 10% defibrinated blood of the dog is added. To obtain the optimum result, the organisms are plated on such a medium and incubated for 16 to 18 hours. On examination with reflected light the "R" colonies present an extremely rough surface in distinct contrast to the smoothness of "S" forms.

Evidences point to the dog blood cells as furnishing the important constituent of such a medium.

5823

Permeability of Muscle to Sodium Ion.

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It is well known that muscles, like blood corpuscles, contain more potassium than sodium, whereas blood plasma contains more sodium than potassium. The unequal distribution of the cations between the corpuscles and the plasma has been shown experimentally¹ and theoretically² to be due to the impermeability of the corpuscle membrane to cations. Although a similar explanation may be offered in the case of muscle, no conclusive proof is available. Mauriac, Aubel and Boutiron³ found that muscle immersed in experimental edema fluid gained Na but lost K, indicating that the muscle membrane is permeable both to Na and K. The per-

* We are indebted to Dr. A. B. Wadsworth of the New York State Department of Health for the antipneumococcus horse serum employed in this investigation.

¹ Doisy, E. A., and Eaton, E. P., *J. Biol. Chem.*, 1921, **47**, 377.

² Wu, H., *J. Biol. Chem.*, 1926, **70**, 203.

³ Mauriac, P., Aubel, E., et Boutiron, *Compt. rend. Soc. Biol.*, 1927, **97**, 78.