Group IV, 70 per cent. The highest incidence for all groups was during the months of January, February, and March, with a decided drop in the late spring and summer months. It is interesting to note that during these four years, no Group II pneumococcus cases, in a series of 40, occurred in the months of June, July, and August. But 12.9 per cent. of the cases (93) belonging to Group I, 8.2 per cent. of cases (110) belonging to Group III, and 8.7 per cent. of the cases (570) belonging to Group IV, occurred during these summer months.

Further analyses of the data mentioned in the foregoing preliminary report are in preparation, the results of which we hope to report more fully later.

179 (1926)

The hemolytic properties of the pneumococcus.

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Recently an interesting phenomenon was observed in culture plates made with the blood from a fatal case of septicemia with meningitis, which followed mastoiditis. The blood culture after 24 hours' incubation showed a considerable number of characteristic green colonies which proved to be pneumococcus, Group IV. One of the culture plates which had been used for demonstration purposes before a class of students was stored in the ice box to be preserved for a later section. Some days later, upon its removal from the refrigerator, it was found that marked zones, simulating hemolysis, had appeared about the colonies, giving an appearance almost identical to that produced by hemolyzing types of streptococci. In our previous experience with pneumococcus blood-culture plates, no hemolyzing effect of this nature had been noted, although no continued observation under similar conditions had been followed. The standard reference- and text-books on bacteriology consulted failed, with one exception, to note that pneumococcus colonies might produce hemolysis in blood-agar plates. Zinsser[1] states that hemolysin production, which occurs

[1] Hiss-Zinsser-Russell, "Textbook of Bacteriology," 1922, 5th Ed., p. 445.

late, is slight but definite with the pneumococcus on blood plates. Brown[2] in his study upon the hemolyzing properties of the streptococcus, ascribes a hemolyzing action to the pneumococcus similar to that shown by his alpha type of streptococcus. As evident, considerable lack of agreement exists among different authorities concerning hemolysin production by the pneumococcus on blood-culture plates. The question remains open for further investigation.

Fortunately, fishings of colonies from this particular strain had been made and cultured upon blood-agar slants. In order to determine if hemolysis is a common property of the pneumococcus, under certain circumstances, tests were performed under varying conditions with this, and other immunological types of the pneumococcus isolated at that time. A series of preliminary tests were first carried out as a guide in studying the problem. The results of these tests showed that under certain conditions the pneumococcus colony produced a hemolysis of the cells in the immediately surrounding blood-agar medium. The principal tests undertaken concerned in particular the question of medium reaction and the influence of temperature upon possible hemolysin production. The medium was prepared from a meat-infusion broth (500 gm. to a liter), to which was added 1 per cent. peptone (Fairchild's), and 2 per cent. shredded agar. The reaction of one portion was adjusted to P_H 7.0 to 7.1, and the other to P_H 8.0 to 8.1. It was tubed in 6 c.c. and 12. c.c. amounts and sterilized in the autoclave at 15 pounds' pressure for 30 minutes. To the agar medium at the time of plating the organism, sufficient freshly drawn, defibrinated, human blood was added to produce a 5 per cent. concentration in Petri dishes (9 cm. diameter), and the whole evenly distributed. By using different amounts of the medium, or slanting the dish, the poured inoculated medium gave layers varying from 0.5 mm. to 3.0 mm. in depth. Pneumococci representing all groups were grown on slanted 5 per cent. human blood beef-extract agar, and used for plating purposes after two to four days' incubation. A small amount of growth was removed and evenly suspended in plain broth. Inoculations were made directly from this into the special agar medium, poured, and mixed

[2] Monographs of the Rockefeller Institute for Medical Research, 1919, No. 9 p. 23.

with the required amount of blood in the Petri dish. Identical platings were made from the same suspension of each organism in the study of the influence of medium reaction (P_H 7.0 and P_H 8.0) upon hemolysin production. For the icebox test, duplicate series of plates, each consisting of both the special neutral and alkaline media, were inoculated in parallel from the same bacterial suspensions, and incubated at 37° C. for approximately 36 hours. Then one series was removed and placed in the icebox at 8° C. for three days. The plates were carefully examined daily, and the results fully recorded.

Only a brief summary of the results of this work can be considered, as the experimental data are too extensive to be recorded in this place. Important factors influencing the clearing or the degree of hemolysis about the colonies were found to be the thickness of the medium layer, and the relative position of the colony to the layer, *i.e.*, on the surface, embedded in layer, or sublayer (between medium and bottom of dish). Surface and sublayer colonies usually were large, but if the medium was rather thick (2 to 3 mm.), they ordinarily showed no surrounding hemolyzed zone. Colonies embedded in the thick layer rarely showed any clearing, but generally, after 36 to 48 hours' incubation, a coloration varying from a deep green, brownish green to almost black. In the thinner layers, they appeared pale green to grayish in color. If a clearing actually occurred immediately in contact with the deeply embedded colony, the outer deep green or brownish green zone of methemogoblin would fully envelope and mask the reaction. Colonies in the thinner layer which showed an immediate inner zone of clearing frequently were surrounded by an outer zone of coloration varying from a pale to a dark, or even brownish green. In general, the colonies which best showed the hemolyzing action were embedded in a blood-agar layer of one mm. or slightly more, and as growth advanced, tended to cause a slight uplifting of the medium. The hemolyzing action, when occurring, usually appeared after 48 hours' incubation, and reached its maximum extent in from 72 to 96 hours. The hemolyzed zone varied from a barely visible clear surrounding ring to one which was 1 or 2 mm. in diameter (occasionally greater), rarely very broad, such as is seen with the hemolyzing streptococcus.

Likewise it very exceptionally produced a similar degree of transparency in surrounding medium. Usually the zone about the pneumococcus colony is translucent, hazy, or of ground glass appearance, and frequently with an outer, more or less diffuse methemoglobin ring, varying in color from pale to brownish green. When examined under the low power of the microscope, the zone of clearing rarely was free from "shadow cells," and in those showing haziness, such cells were much more in evidence. If the zone appeared slightly opaque or pigmented, the blood cells showed certain amounts of the changed hemoglobin or methemoglobin present. The zone of clearing about the pneumococcus colony can hardly be considered a hemolysis in the sense as applied to that produced by the *Streptococcus hemolyticus*. The first is probably due to an intracellular hematoxin, liberated by autolysis of organisms in the colony, while the second is probably extracellular, elaborated and passed out by the living organism. In the case of the pneumococcus, perhaps it would be more appropriate to designate the change as a pseudo-hemolysis, since it lacks the completeness of action shown by the hemolytic streptococcus. Further, the reaction is probably complicated by two independent processes occurring at the same time—methemoglobin formation induced by the living organisms, and a hemolysis produced by a hematoxin arising from autolyzed cells, as suggested by Cole's studies on pneumococcus hematoxin,[1] and methemoglobin production by the pneumococcus.[2]

The hemolyzing action of different pneumococcus strains within the same group was found rather irregular; certain ones appeared to possess that ability to a greater degree than others. Even the same strain on repeated tests showed considerable variation.

The medium reaction (P_H 7.0 and P_H 8.0) in which the organisms were plated produced no apparent variation in growth or hemolytic action at incubator temperature. Also no appreciable difference in hemolysis could be recognized in a similar series of plate cultures, which were placed in the icebox three days after a primary incubation of 36 hours. Methemoglobin production was

[1] *Jour. Exper. Med.*, 1914, xx, 346.

[2] *Jour. Exper. Med.*, 1914, xx, 363.

inhibited in the icebox, but progressed in the controls which were left in the incubator. No inhibition to hemolysis, as occurs with the *Streptococcus hemolyticus*, was observed with the pneumococcus plated in a medium (P_H 7.8) containing 1 per cent. dextrose. These plates were first incubated three days, then stored in the icebox three days.

Fishings of pneumococcus colonies which showed markedly clear zones in blood plates were cultured on blood-agar slants. When replated these cultures produced colonies which failed to show any pronounced differences from the original cultures.

The hemolysis probably depends, among other factors, on depth of agar layer, the percentage of blood corpuscles present, and the age of the colony, and its vitality. A number of other possible factors might enter this reaction which cannot be discussed in this paper, but we hope that others may take up this problem more fully and investigate the question.

CONCLUSIONS.

In conclusion, our results would indicate that, (*a*) pneumococci of all serological groups, under certain cultural conditions, may hemolyze human erythrocytes, and, (*b*) apparently, this property is not influenced by the reaction of the medium within the growth limits of the organisms, nor (*c*) by prolonged refrigeration of the developed colonies on blood-agar plates. (*d*) Probably the hemolysin is an intracellular product liberated from autolyzed organisms which diffuse from the colony into the surrounding blood agar.

180 (1927)

Studies on the therapeutic effect of B. acidophilus milk and lactose.

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In a series of psychotic and normal (mentally) subjects relief from chronic constipation and diarrhea was obtained by the inges-