

of the various types is so little understood, and the morphology so inconstant, that classifications based on these characters alone are necessarily incomplete, and even apt to be misleading.

Later classifications, however, depend upon the reactions of streptococci in various culture media, and have as their basis the power possessed by these organisms of hæmolyzing red blood cells, and fermenting certain carbohydrate radicles. These cultural characters are, in our experience, constant, and bear a definite relation to certain serological reactions; they also have the advantage of being readily studied.

By their growth on blood agar to which no "sugar" has been added, streptococci are divided into two primary groups, hæmolytic and non-hæmolytic. The colonies of the former are characterized by the general absence of pigment and by the presence of a sharply defined encircling zone, free from hæmoglobin and red blood cells, the latter by the absence of such a zone and the presence of pigment. A narrow zone of hæmolysis is sometimes seen around the colonies of non-hæmolytic strains, but this can hardly be confused with the sharply defined clear zone produced by hæmolytic strains, for the zone is much smaller (narrower and shallower), the destruction of red cells is less complete, and a large number of them are seen immediately beneath the non-hæmolytic colonies, while few, or none at all, are seen beneath the hæmolytic ones. These points of difference can be recognized readily by using the low power of the microscope. A satisfactory method of demonstrating them is as follows:—

Punch out with a cork borer a cylinder of agar, including a hæmolytic colony, its clear zone, and a portion of the surrounding media; with a sharp safety razor blade make a thin vertical section through the colony and the entire thickness of the underlying agar. Lay the section flat on a clean glass slide, and examine it with a hand lens and the low power. Compare this with a similar section made through a non-hæmolytic colony.

The factors concerned in the production of the hæmolytic zone are not fully understood. This phenomenon has been said to depend upon the action of a hæmolysin, but of this we know but little, nor do we understand how the freed hæmoglobin and the bodies of the red cells are disposed of. It seems reasonable to suppose that they are not merely destroyed, but made use of in the metabolism of the organism.

The majority of the non-hæmolytic strains produce colonies of various shades of brown or green, and this occurs more often on blood agar containing 1 per cent. glucose. The pigmentation is due to methæmoglobin, but how it is produced we do not know. It has been suggested that it is formed from hæmoglobin by the action of acids derived from the carbohydrate in the media. This seems improbable, as streptococci grown in dextrose ascitic bouillon to which sheep's corpuscles have been added produce methæmoglobin very readily, even when the neutrality of the medium is maintained by the addition of secondary phosphates.

As some non-hæmolytic strains are not pigmented, Mandelbaum, Le Blanc, Lyaill and others have suggested that they should constitute a group by themselves. By their reactions upon blood agar streptococci would then be divided into three groups: hæmolytic, methæmoglobin producers, and "indifferent." The members of the suggested third primary group, however, are so closely allied to the methæmoglobin producers with regard to their pathogenicity, distribution in nature, morphological and cultural characters, that it would be misleading to place them in a class by themselves. It has also been shown that the serum of animals, immunized against one member of the hæmolytic group, will agglutinate other hæmolytic strains, but will not agglutinate members of the non-hæmolytic group, whether they be methæmoglobin producers or not; cross-agglutination, however, occurs between methæmoglobin producers and "indifferent" strains.

The ability of streptococci to ferment certain "sugars," or the lack of this property, is now largely used as a basis for the determination of subdivisions of the two main groups (hæmolytic and non-hæmolytic). The number of "sugars" used by different investigators has varied from few to many, and within certain limits the number of subdivisions increases with the number of "sugars" employed. Important as the fermentation reactions are in determining subdivisions of the two main groups, they cannot be used alone as means of identifying all the known strains of streptococci, as some

members of the hæmolytic group give the same sugar reactions as some of the non-hæmolytic group. To give an example, *Streptococcus pyogenes* (hæmolytic) and *S. mitis* (non-hæmolytic) ferment saccharose, lactose, and salicin, but not mannite, raffinose, nor inulin; there is, however, no cross-agglutination between these organisms, and the former is considered to be much more virulent than the latter.

Much of the difficulty in analysing the records of earlier workers, and comparing them with those of later investigators, must be ascribed to too great a reliance on sugar reactions as a means of differentiation, and the failure to make good use of the blood agar plate.

Classifications comprehensive enough to include all known streptococci are of the greatest importance, their value increasing as our knowledge of specific therapy advances; for from our collective knowledge of these organisms it is possible to form more rational conclusions with regard to their pathogenicity, the sources from which they are derived, as well as the treatment and prognosis of any given case. Beside the more comprehensive classifications others have been suggested, primarily with the view of separating the more pathogenic groups from the less pathogenic ones, rather than identifying the various members of each group. These "clinical" classifications are of assistance to the surgeon and physician, and at present fulfil the requirements of a routine laboratory examination.

Research on streptococci has done more than simply point out differences between closely allied members of a widely distributed and very important group of organisms; it has paved the way for more accurate knowledge based on serological reactions, and incidentally thrown some light upon the biology of bacteria in general.

During the past year we have been studying the streptococci isolated from various types of wounds, and the relation of this group of organisms to war wounds in general, and to their surgical complications.

The work was undertaken primarily with the hope that we might be able to assist the surgeon in forming an opinion with regard to the proper surgical procedure, subsequent treatment and prognosis in cases of streptococcal infection, for all of these depend in some degree upon the particular type of infecting organism.

For this report we have selected for analysis twenty-five of the cases studied. In these the lesions chosen for special study bore an indirect connection with the track of the missile and with the outside, or contained streptococci in pure, or nearly pure, culture. Such an indirect connection is seen, for example, in a shrapnel wound of the leg, where there is only bloody effusion in the knee-joint, without a fracture into the joint itself, or laceration of the capsule. The abscesses included in this report developed at such a time after the primary injury, or bore such a relation to it, as to warrant a conclusion that they were secondary infections. In other cases, as in the thoracic injuries, the wounds were closed, and streptococci were recovered in pure, or nearly pure, culture.

By such a selection of cases we hoped to determine the type, or types, of streptococci that lead to surgical complications of wounds rather than those found in the track of the missile, its lining, or the tissues immediately about it; further, we hoped to find streptococci in pure, or nearly pure, culture, or in such numbers as to be able to attribute the lesion to them. This would be difficult in cultures made from the track of the wound soon after injury.

The classification we have adopted is that suggested by W. L. Holman, and while we hold no brief for the system of nomenclature employed, this classification is comprehensive enough to include over 2,400 strains of streptococci, 50 per cent. of which are hæmolytic.

HÆMOLYTIC.	Lactose.	Mannite.	Salicin.	NON-HÆMOLYTIC.
<i>S. infrequens</i>	+	+	+	<i>S. faecalis</i>
<i>S. hæmolyticus</i> (i)	+	+	—	<i>S. non-hæmolyticus</i> (i)
<i>S. pyogenes</i>	+	—	+	<i>S. mitis</i>
<i>S. anginosus</i>	+	—	—	<i>S. salivarius</i>
<i>S. hæmolyticus</i> (ii)	—	+	+	<i>S. non hæmolyticus</i> (ii)
(iii)	—	+	—	" (iii)
<i>S. equi</i>	—	—	+	<i>S. equinus</i>
<i>S. subacidus</i>	—	—	—	<i>S. ignavus</i>

+ = Fermentation with acid production.

— = No fermentation.