HÆMOLYTIC STREPTOCOCCI IN PUERPERAL SEPSIS

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HIGH vaginal swabs are sent to this Department from women showing pyrexia during the puerperium in order to determine whether group A hæmolytic streptococci are present, this group being the most virulent and the cause of hospital epidemics of puerperal sepsis and septicæmia.

Only 51 per cent. of the beta-hæmolytic streptococci isolated over a period of two years were found to be group A. The opportunity was therefore taken of determining the incidence of groups B, C and G streptococci in these cases, while beta-hæmolytic enterococci—whose antigens are similar to those of Lancefield group D—were also tested against D grouping sera.

These beta-hæmolytic streptococci, *i.e.* those showing a hæmolytic zone on blood agar plates, were tested also for soluble hæmolysin (using washed human cells), for fibrinolysin and as to their biochemical reactions.

One hundred strains from high vaginal swabs were isolated and examined, while the results obtained with 167 other beta-hæmolytic strains from various sources, chiefly throat swabs, are shown in the chart for purposes of comparison. The high vaginal swabs were taken from patients showing degrees of puerperal sepsis varying from trivial to severe.

METHODS

The high vaginal swabs were plated out on (human) blood agar plates and incubated overnight. Streptococcal colonies showing a zone of hæmolysis were subcultured on to laked blood agar (human blood was used in the preparation of this medium) and incubated for six hours and further subcultured in a 1 per cent. glucose trypsin broth, and in 10 per cent. serum peptone water. The resultant growth in the 1 per cent. glucose trypsin broth was centrifuged, and from the sediment an antigen was prepared for the precipitin test according to the Lancefield technique.

Fibrinolysin (Tillet and Garner).—Human plasma was obtained by collecting 5 c.c. of blood into a tube which had been previously prepared by evaporating 0·5 c.c. of a 2 per cent. potassium oxalate in the hot air oven. The blood was mixed by gently rolling the tube and centrifuging to obtain the plasma. For the test the following materials were mixed: 0·8 c.c. of N/1 saline, 0·2 c.c. of oxalated plasma, and 0·5 c.c. of the 10 per cent. serum peptone water culture; coagulation was effected by the addition of 0·25 c.c. of a 0·25 per cent. solution of calcium chloride, and incubation in a waterbath at 37° C. The clot formed within a short time after the addition of the calcium chloride. Strongly fibrinolytic strains should dissolve this clot within fifteen minutes.

Hæmolytic Suspension.—Oxalated human blood was repeatedly washed with N/1 saline till a clear supernatant was obtained, and a 2 per cent.

suspension of the washed red blood cells was made in N/1 saline. For the soluble hæmolysin test 0·5 c.c. of the suspension was mixed with 0·5 c.c. of a 10 per cent. serum peptone water culture and incubated at 37° C. in a waterbath. The result was read after two hours.

Biochemical Reactions.—Hiss's serum water medium containing 1 per cent. of the various sugars was used with a 1 per cent. Andrade's solution as indicator.

Results

Forty-eight per cent. of the 100 strains from high vaginal swabs proved to be group A, 16 per cent. group B, 10 per cent. group C, 8 per cent. group G, 9 per cent. group D, and 9 per cent. did not fall into any of these groups.

Group A.—In most of the cases yielding group A streptococci there was a heavy growth, and only 25 per cent. showed other organisms, staphylococci and *B. coli* being the most common. The zone of hæmolysis surrounding the colony was invariably large and well defined, and a soluble hæmolysin was produced with the human red cell suspension. All the streptococci isolated in this group were fibrinolytic, three hours being the average time taken to dissolve the clot. The majority of these strains fermented the usual sugars as shown in the chart, trehalose being fermented but not sorbite. Two strains, however, fermented both mannite and sorbite, and one fermented mannite but not sorbite. Starch and glycogen were fermented by 6 strains.

Group B.—Of the 16 group B strains only 56 per cent. showed a heavy growth, 63 per cent. being accompanied by other organisms. The zone of hæmolysis was very much smaller, only about 12 per cent. having a zone of hæmolysis comparable with those in group A, and all the strains failed to produce a soluble hæmolysin with the human red cells suspension. A sheep cell suspension, however, was hæmolysed to a considerable extent with all the strains. The fibrinolytic test was negative with all strains after eight hours, at which time it was arbitrarily terminated.

The lack of true hæmolytic and fibrinolytic activity suggests a low virulence for human beings, but these strains resembled the A group in fermenting trehalose but not sorbite.

Group C.—A good growth was obtained in 5 of the 10 cases, but only in 3 was there a pure culture. The zone of hæmolysis was very marked, in some instances being larger than those of group A, and occasionally (4 cases) the clear zone diffused into the surrounding medium giving the appearance of having a scalloped margin. A soluble hæmolysin was produced by all the strains, and only one was not fibrinolytic. The time taken to dissolve the clot was slightly less than that with group A. Biochemical reactions were again regular, trehalose being fermented and sorbite negative, suggesting that the strains were of human origin. Starch and glycogen were fermented by all the strains of this group.

Group G.—In 4 of the 8 group G cases a strong growth was

TABLE

Group.	Source.	Strong Growth.	Pure Growth.	Strong Hæmolysis. (Plate)	Hæmolysis. (Suspension)	Fibrinolysis.	Dextrin.	Galactose.	Glucose.	Glycogen.	Inulin.	Lactose.	Maltose.	Mannite.	Mannose.	Raffinose.	Saccharose.	Salicin.	Sorbite.	Starch.	Trehalose.	Gelatin.	MacConkey's Medium.
A.	48 HVS.	46	36	48	48	48	30	43	48	6	0	44	47	3	48	0	47	41	2	6	48	...	...
	88 Mis.	68	29	85	88	86	59	80	87	11	0	80	86	12	87	3	88	62	8	11	88	...	...
B.	16 HVS.	9	6	2+++ 14+	0	0	12	11	16	0	0	16	16	0	16	0	16	11	0	0	16	...	...
	3 Mis.	1	2	1+++ 2+	0	0	3	3	3	0	0	3	3	0	3	0	3	3	0	0	3	...	...
C.	10 HVS.	5	3	10	10	9	9	10	10	10	0	10	10	0	10	0	10	9	0	10	10	...	...
	6 Mis.	5	0	6	6	5	6	6	6	6	1	6	6	0	6	2	6	5	1	6	6	...	...
D.	9 HVS.	3	1	0+++ 9+	0	0	9	8	9	0	0	9	9	9	9	0	9	9	9	0	9	1	9
	8 Mis.	5	5	4+++ 4+	0	0	6	5	8	0	0	5	8	4	8	0	6	7	4	0	8	0	8
G.	8 HVS.	4	3	8	8	4	8	8	8	8	0	7	8	0	8	0	8	5	0	8	8	...	...
	1 Mis.	0	0	1	1	0	0	1	1	0	0	1	1	0	1	1	1	1	0	0	1	...	...
X.	9 HVS.	3	1	0+++ 9+	0	0	8	5	9	1	3	5	5	4	9	4	8	8	4	2	5	...	0
	61 Mis.	18	1	8+++ 53+	0	0	36	56	58	2	10	47	40	2	58	24	61	19	1	6	11	...	...

HVS. = high vaginal swabs. Mis. = other sources. X group = not of groups A, B, C, D, or G.

obtained, and on three occasions the hæmolytic streptococcus was the only organism isolated. The zone of hæmolysis was similar to that of groups A and C. A soluble hæmolysin was produced in each instance. Only 4 of the strains, however, were fibrinolytic. The biochemical reactions were fairly constant, with starch and glycogen fermented on every occasion. Again trehalose was fermented but not sorbite.

Group D.—Enterococci were isolated on numerous occasions but only 9 showed an area of hæmolysis, which was never marked, surrounding the colony. In only 3 instances was the growth heavy and in one case a pure growth was obtained. A soluble hæmolysin was never produced by any of the strains. They all grew on MacConkey medium; gelatin was liquefied by one strain. Biochemical reactions were constant, mannite and sorbite being fermented by all the strains. The reaction with the grouping sera was very slow in appearing, three hours being the average time for the precipitate to appear.

Other Groups.—Nine strains did not fall into any of the above groups. Three of them yielded a good growth, which was pure on one occasion. The zone of hæmolysis was very slight; a soluble hæmolysin was never produced and the fibrinolytic test was also negative. They did not grow on MacConkey medium. The biochemical reaction varied considerably.

Controls

Beta-hæmolytic streptococci from various sources but mostly from throat swabs are also included in the chart. Most of them reacted in the same way as the strains isolated from the high vaginal swabs. For instance the percentage of 12·5 of group A strains fermenting starch and glycogen was the same both in the control series and in those from the high vaginal swabs.

Group B Controls.—Only one strain of group B was isolated from a throat swab, that being from a scarlet fever contact. As the individual drank unpasteurised milk this would be the probable source. Further swabbing produced a group A streptococcus. The other 2 strains in this group were isolated from the genital tract.

Group C Controls.—Six throat strains all fermented starch and glycogen. One strain fermented inulin and sorbite as well as trehalose.

Groups G and D Controls.—All had the same characteristics as the high vaginal strains.

Clinical Features

A clinical analysis is outwith the scope of this investigation, but details were obtained of the duration of stay in hospital of most of the cases. Many of the group A cases were transferred to the City Fever Hospital, as soon as it was known that their infection was of this kind; their transfer was not necessarily indicative of a severe

infection. Of the group B cases, one was transferred to the City Hospital and of another there was no record. Fourteen cases had an average duration in hospital, after the onset of fever, of 11 days. Group C, eliminating one sent to the City Hospital, and two with no record, had for 7 cases an average stay of 16 days. Group D, omitting one with no record, had an average stay of 12·5 days for 8 cases. Group G cases, with one to the City Hospital, had an average stay of 10 days for 7 cases. Of the cases yielding hæmolytic streptococci not falling into any of these groups, two had no record, and one was transferred to the City Hospital; the average stay of the remaining 6 cases was 6 days.

So far as this evidence goes, therefore, it suggests that group C infections were, after group A, the most severe, and the ungroupable streptococci the least severe; with groups D, B, and G cases occupying intermediate positions.

Conclusions

The formation of a soluble hæmolysin by the streptococci of groups A, C and G is evidence of the presence of an exotoxin, while the positive fibrinolytic test with 100 per cent. of the group A strains, 90 per cent. of the group C and 50 per cent. of the group G is proof of the invasive power of these strains.

The power of the groups C and G strains, like those of group A, to ferment trehalose and not sorbite is further evidence of their human origin. The fact that in 3 of the group C and 3 of the group G cases the growth of streptococcus was a pure one is corroborative evidence of ætiological connection between the organism and the condition of sepsis. The evidence for the pathogenicity of the groups B and D strains is not so strong, with an absence of a strong hæmolysin and of fibrinolytic activity. Nevertheless the presence of these strains in pure culture in six instances with group B and one with group D suggests that they may cause a lower grade of sepsis. One strain not falling into these five groups was also obtained in pure culture.

Fermentation tests with trehalose and sorbite are helpful in differentiating between "human" strains of groups B and G from those of animal origin. Starch and glycogen were found in this series to have a limited use in differentiating between group A and groups C and G.

While group A streptococci are the undoubted important bacteriological factor in severe puerperal sepsis and septicæmia, streptococci of groups B, C, D and G are also associated with an appreciable number of cases in which the sepsis is usually less severe. Of these, group C cases had the longest stay in hospital.

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Reference

Tillet, W. S., and Garner, R. L. (1933), *Journ. Exp. Med.*, **58**, 485-502.