STREPTOCOCCUS VIRIDANS IN URINE.

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In spite of an extensive literature recording the great amount of investigation which has been carried out on the Viridans (a) group of streptococci it is by no means clear to what extent they are to be considered pathogenic, and to what degree and where they are saprophytic; in other words, where in the human body their presence may be ignored.

Okell and Elliott (1935) have demonstrated the "almost physiological entry" of Str. viridans into the blood-stream postulated by Lewis and Grant, both after dental extractions and even without extractions in certain cases with septic mouths, thus casting doubt on the powers of these organisms to cause endocarditis in a previously healthy heart. This work has been confirmed by Fish and Maclean (1936) who show that the original focus in such cases is the gums and not the living tooth pulp as was previously thought to be the case. The latter also quote two fatal cases of malignant endocarditis following tooth extractions in patients with mitral disease.

Rosenow (1914-1928), the originator of the well-known "Elective localization" theory, considered that localized dental infection (with α streptococci) was responsible for a number of different clinical diseases. His work has gained a certain amount of corroboration, but has also excited much criticism.

Williams (1932) sums up the general situation as follows: "The part played by these α and γ (non-hæmolytic) types so frequently and, in certain localities, so abundantly present in the normal individual has been much studied, but their relationships, their power to dissociate and cause pathological changes of various sorts are only just beginning to be known."

References to streptococci in the urinary tract do not appear to be extensive.

1 The symbols α, α' (alpha prime) and β are used in this paper to designate the types of hæmolysis in blood agar produced by streptococci. (Smith and Brown, 1915; Brown, 1919.)

α type.—There is a zone of un-hæmolysed cells and greenish discoloration round colonies, surrounded by a partially hæmolysed zone. Alternate incubation and refrigeration produces multiple concentric zones of hæmolysis and blood-cells.

β type.—Each colony is surrounded by a perfectly clear colourless zone of hæmolysis. There is no change on refrigeration.

α prime (α') type.—This is superficially like β hæmolysis, but there is a narrow zone of non-hæmolysed corpuscles around the deep colonies visible under the microscope. The zones enlarge during refrigeration, though multiple zones are not produced.
Rosenow includes nephritis and nephrolithiasis among the conditions which he considers primarily due to dental infections.

Williams includes urethritis in a list of chronic conditions in which \( a \) and \( \gamma \) streptococci have been found.

Longcope (1936) records high antistreptolysin titres obtained in certain cases of acute haemorrhagic nephritis but rejects this as evidence that the condition is caused by hemolytic streptococci, believing that such titres merely indicate the recent occurrence of an infection with these organisms.

Heckell and others (1936), however, using a special technique, isolated \( Str. \ viridans \) from the prostate, urethra or “bladder urine” in fourteen of seventeen cases of urinary tract infections. They conclude that such streptococci are frequently present in genito-urinary tract lesions, that serological studies frequently show them to be primary invaders, though in some instances they may be secondary, and that “their presence in solitary ulcer (of the bladder), so-called sterile pyuria, prostatovesiculitis, and non-specific urethritis is difficult of explanation except to view them in the role of infecting microbes.”

Normal controls are not mentioned.

**Present Investigation.**

The object of this investigation was to discover if the presence of \( Str. \ viridans \) in the urine is of any pathological significance.

The technique was as follows:—

**Tests.**—Urine, which was collected with sterile precautions, preferably by catheter, and defibrinated rabbit’s or human blood were added to melted agar at \( 55^\circ \) C., plates were poured and the cultures incubated at \( 37^\circ \) C.

**Controls.**—To ensure that any growth observed was not due to contamination (or infection) in the blood used: A. Two or more plates were poured for each specimen, using varying amounts of urine (indicated either as ++, +, and +, or by definite volume, e.g. 5, 2 and 0.5 cubic centimetres), to observe if growth was proportional to the amount of urine in culture. B. Uninoculated plates of the same batch were tested by incubation.

Either A or B or both controls were used in all cases.

**Cultures from Cases.**

The results of urine culture by this method in thirty-four cases are shown in Table I, A, B and C. It will be noted that with one possible exception absolute sterility was never found. This, in view of the severity of the test, is not surprising. The figures in the table represent cases, not the number of specimens—56 specimens were tested, the number from each case varying from 1 to 6.

**Streptococci.**

\( Str. \ viridans \) was present in pure culture in four cases, or 12 per cent, and with other organisms in ten cases or 30 per cent.
In nine of the cases catheter specimens were examined. Three of these were in addition to "sterile" specimens, while six were the only specimens examined in the cases concerned. Of the former, two were positive for Str. viridans and one negative, Str. viridans being isolated from "sterile" specimens in all three. The result of the examination of these nine specimens is shown in Table II.

**Description of Streptococci Isolated.**

The morphology and cultural reactions of the cocci isolated were examined in several cases. In fifteen instances recorded the chains were very long and often tangled. It was frequently noticed that the cocci were pleomorphic, being elongated and even bacillar in appearance as seen in chains, while in certain instances from some media the chain appearance was lost and the organisms appeared like bacilli. In this respect they resembled the "pleomorphic streptobacillus" described by Stuart-Harris and others (1935). Such pleomorphism is common, however, with Str. viridans (Williams, 1932). Chains of moderate length are recorded in four instances. Inulin fermentation was studied in 13 strains: It was positive in 7, variable in 2 and negative in 4. In the case of the organisms exhibiting long tangled chains growth in broth was usually clear with a floccular deposit. These two characteristics were probably rather associated with "roughness" and virulence as described for Str. haemolyticus (Hadfield and others, 1934), than indicative of a definite species. Haemolysis: The haemolytic activity of certain strains was examined in some detail. The organisms from two cases showed α haemolysis (at first considered to be β) on human blood-agar, but α haemolysis on rabbit blood-agar. Culture in blood-broth of one of these strains showed no haemolysis. This organism was agglutinated by antipneumococcal serum to 8 per cent of the titre of a Group I serum and 40 per cent of the titre of a Group III serum. Culture in human blood-broth of the other strain showed slight haemolysis in some tubes.

The phenomenon of certain strains reacting differently to blood-cells from different animals is referred to by Williams, who mentions that Cummings (1927) gave the name Str. pseudo-haemolyticus to strains he found that did not haemolyse rabbit's red cells, and quotes Brown's (1919) description of a strain which gave α haemolysis in horse blood-agar and a haemolysis in rabbit blood-agar.

The strain from a third case which appeared to be Str. viridans (α haemolysis) in plates produced haemolysis in human blood broth, while a fourth organism produced apparently true β haemolysis in human blood agar and α haemolysis in rabbit blood agar; culture in human and rabbit blood broth of this strain showed slight haemolysis in 4 of 8 tubes of human blood but none in rabbit blood. In no case, however, were haemolytic tests carried out with washed cells or using culture filtrates.
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Biochemical Reactions.

The Sugar Reactions were Inconstant.—Lactose was fermented by all but 2 of 12 strains tested. Glucose was fermented by all of 11 strains tested. Saccharose was fermented by 8 of 11 strains tested, 1 of the others being variable with 2 negative. Fermentation of dulcite was only tested 3 times, all strains being negative.

Mannite, used 8 times, gave 6 negative results and 2 variable. The reactions with inulin have already been described.

Thus the majority of strains examined appear to fall within Brown's subgroups 3 and 4 of either Str. mitis or Str. salivarius (Andrews and Gordon).

Salicin fermentation to differentiate between these two groups was not investigated.

TABLE I.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cases</th>
<th>No. showing Str. viridans in pure culture</th>
<th>No. showing Str. viridans + other organisms</th>
<th>No. showing Str. fecalis</th>
<th>No. showing other organisms without streptococci</th>
<th>Sterile</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>14</td>
<td>3</td>
<td>5 (1) (2)</td>
<td>2</td>
<td>3</td>
<td>? 1</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>12</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Notes.—(1) In one case from only one specimen of six examined, but four were obscured by a growth of Proteus.

(2) In one case Str. viridans was twice present in large numbers in "sterile" specimens but a ureteric catheter specimen produced no growth.

TABLE II.—Results of Examination of a Catheter Specimen from Cases Included in Table I.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cases</th>
<th>Str. viridans in pure culture</th>
<th>Str. viridans + other organisms</th>
<th>Str. fecalis</th>
<th>Other organisms (no strep.)</th>
<th>Sterile</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>1</td>
<td>2 (1)</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

(1) In one case "other organisms" were Str. fecalis.
OTHER ORGANISMS.

Other organisms commonly isolated were diphtheroids (9 cases) and staphylococci—often with the diphtheroids, 17 cases.

The predominant organisms isolated from two cases in Group A exhibited the morphological and cultural characteristics of the genus *Hemophilus*. One species of this genus, *H. canis* was originally isolated from the prepuce of dogs. The organisms I isolated, however, were haemolytic, while *H. canis* was not.

CONTROL CULTURES.

In view of the results shown in Table I, A, B and C, it was obviously desirable to make cultures employing the same technique from the urine of normal individuals. This was done in 4 cases. It occurred to me also that with a view to eliminating organisms derived from the anterior urethra—a very probable source in spite of the not infrequent positive culture from catheter specimens—cultures should be taken from cases of gonorrhoea towards the end of a course of irrigation, the specimen collected being the first urine passed after an irrigation. This was done in 5 cases, the results of each group being shown in Table I, D and E. The groups are very small so that the negative result for *Str. viridans* in Group E cannot be accorded much significance, but it is possibly worth remark that cultures from 3 of these 5 cases were positive for *S. faecalis* as against 5 (3 as shown in Table I and 2 others occurring with *Str. viridans* and not fully investigated) positives from the 38 individuals in the other groups.

It will be noted that so far there is nothing to suggest that *Str. viridans* isolated from urine has origin other than from the urethra. In a case of adenitis of the groin, suspected as originating from a urethral or meatal infection, a smear from the meatus showed epithelial cells, diplococci and short chains. Two cultures from the apparently dry meatus (one made without cleansing and the other after washing the glans with spirit) both produced a growth of very long chain streptococci. *Str. viridans* was not plentiful in any of the catheter specimens examined, and such growth as was observed might occur as the result of contamination of the catheter in passing.

URINE AND BLOOD CULTURE FOLLOWING DENTAL EXTRACTION.

I thought it of interest, however, to repeat on a small scale the experiment of Okell and Elliott (1935)—who obtained positive blood cultures for *Str. viridans* after dental extractions—examining in addition the urine both before, and at a considerable interval (usually twenty-four hours) after, extraction. The result of such examination in seven cases is shown in Table III. The series is again very small but shows a complete lack of correlation between positive blood and urine culture in these cases.
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**TABLE III.—DENTAL CASES.**

<table>
<thead>
<tr>
<th>Urine Culture</th>
<th>Urine Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Str. viridans both before and after extractions</td>
</tr>
<tr>
<td>Cases having positive blood culture for Str. viridans after extractions</td>
<td>4</td>
</tr>
<tr>
<td>Cases having negative blood culture for Str. viridans after extractions</td>
<td>3</td>
</tr>
</tbody>
</table>

Note.—In no case was Str. viridans absent or scanty in the urine before extraction and present or notably increased on culture after extractions.

**EXPERIMENTS WITH RABBITS.**

I now attempted to discover if the inoculation of living streptococci in large doses intravenously in the rabbit led to the appearance of these organisms in the urine.

**TECHNIQUE.**

Fifteen animals were used. They were divided into four series, according to the source of the inoculum used. Each animal in the series was inoculated intravenously with the same dose (calculated by opacity in series 2, 3 and 4) of streptococci suspended in saline. It was either anaesthetized or killed at a fixed time after inoculation, the chest and abdomen opened, blood and urine obtained in a sterile syringe by heart and bladder puncture, and cultures made. The results are shown in Table IV.

From the 15 animals used, blood culture was positive in 8, and urine cultures in 2.

Thus it appears that with large doses of streptococci liberated into the blood a streptococcal bacteriuria may occur in the experimental rabbit. Finally, to ascertain if there was any selective concentration of organisms in the kidneys, known proportions of the kidneys of the rabbits in series 3 and 4 were removed with aseptic precautions, ground up in a sterile mortar with sterile sand, suspended in a known volume of sterile saline and cultured in a series of dilutions in saline. In the case of series 3 the spleen was similarly treated as a control, being the organ where one would most expect bacterial concentration to occur. The inoculum, in addition to being counted by opacity, was submitted to a viable count by culture of serial dilutions for comparison with the serial dilution cultures of the organs.

The animals in series 3 showed a rapid diminution of the organisms in the blood and spleen, as the time between inoculation and examination was increased, while the numbers in the kidney remained relatively steady. At first the concentration in the spleen greatly exceeded that in the kidneys,
but later was lower (if no allowance is made for the relative volume of the organs). A point of interest is the remarkable difference in the total and viable counts of the inoculums used. That in series 3 being in the approximate ratio of 1:5 would be readily accounted for by chain length,

<table>
<thead>
<tr>
<th>Series</th>
<th>No. of animals in series</th>
<th>Blood before inoculation</th>
<th>Inoculum</th>
<th>Intervals between inoculation and examination (one rabbit at each time)</th>
<th>Blood after</th>
<th>Urine positive on culture for streptococci</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>All sterile</td>
<td>Mixed emulsion: short strep. - from blood (of Dental Case 9); long strep. - from urine (Case 10)</td>
<td>2, 4, 7 and 24 hours</td>
<td>+ + after 7 hours</td>
<td>+ (11 of 16 colonies were short chain strep.) after 24 hours</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>Slight contamination in each case</td>
<td>945 million Str. viridans from throat</td>
<td>12, 18, 37 and 61 hours</td>
<td>+ (single colony) after 12 hours</td>
<td>+ at other intervals</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>3 sterile, 1 contaminated</td>
<td>1,750 million Str. viridans from tooth root (Case 6)</td>
<td>5, 12, 24 and 48 hours</td>
<td>+ + + + after 6 hours</td>
<td>+ at other intervals</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>All contaminated</td>
<td>450 million Str. viridans from throat</td>
<td>24, 72, 120 hours</td>
<td>+ + after 24 hours</td>
<td>+ at other intervals</td>
</tr>
</tbody>
</table>

but that in series 4 being in the ratio of 1:200 cannot be so explained, and probably, together with the longer intervals used, accounts for the failure of all cultures from the animals in this series.

SUMMARY AND CONCLUSIONS.

(1) Culture of the urine in blood-agar was carried out in 14 cases with symptoms suggesting disease of the urinary tract; Str. viridans was isolated from 8.

Using the same technique, 6 of a group of 20 cases without definite history of urinary symptoms gave positive cultures for these organisms; of 4 healthy controls 3 yielded a positive result; cultures from 5 convalescent cases of gonorrhoea taken following urethral irrigation were all negative for Str. viridans, though Str. faecalis was isolated from 3 cases; and 4 of 7 individuals produced positive cultures both before and after dental extractions, there being no relationship between positive blood and urine cultures for Str. viridans in this series.
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(2) The habitat of these organisms is probably the urethra, though animal experiment suggests that in the event of a massive invasion of the blood-stream (as occurs in similar circumstances with others, and as is postulated for B. coli by Evans, 1935), they may be passed in the urine.

(3) Other organisms frequently isolated under similar conditions are Staphylococcus albus and diphtheroid bacilli.

(4) It is considered that Str. viridans in the urine is without pathological significance.

Acknowledgments.

I wish to express my thanks to Major W. A. Cowden, Army Dental Corps, for his collaboration in the investigation of the dental cases, and to my laboratory assistants, Private J. W. Allen, R.A.M.C., and Corporal R. E. Young, R.A.M.C., who afforded me great help in this work.

References.