A FURTHER NOTE ON THE ANTIGENIC RELATIONSHIPS OF DONOVANIA GRANULOMATIS (ANDERSON)

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In previous papers (Dunham and Rake, 1948; Rake, 1948) it has been pointed out that a certain percentage (14 per cent) of sera from individuals with chronic superficial nonspecific ulceration (decubitus or varicose) show positive and apparently specific complement fixation with antigen prepared from Donovania granulomatis, the etiological agent of granuloma inguinale. It has also been shown (Rake, 1948) that sera from individuals with granuloma inguinale show positive and apparently specific complement fixation with antigen prepared from Klebsiella pneumoniae in the same manner as that used for preparation of the antigen from D. granulomatis. It was suggested at that time that the results indicated antigenic relationship of the new genus Donovania to Klebsiella, and further exploration of possible relationships within the tribe Escherichae was promised (Rake, 1948). The apparent false positive complement-fixation reactions in individuals with chronic ulceration were thought to depend on secondary infection of their wounds with organisms showing such antigenic relationships (Rake, 1948). In the present paper results are presented with antigens prepared from Klebsiella rhinoscleromatis, Escherichia coli, and Aerobacter aerogenes, and tested in the complement-fixation test along with antigens from D. granulomatis and K. pneumoniae.

MATERIALS AND METHODS

Preparation of the antigens from D. granulomatis and K. pneumoniae have been described elsewhere, as has the technique of the complement-fixation test employed (Dunham and Rake, 1948; Rake, 1948). The antigens from K. rhinoscleromatis, E. coli, and A. aerogenes were prepared from the sedimented bacterial bodies in the manner described for antigen FB obtained from K. pneumoniae as described elsewhere (Rake, 1948). They were used in the test at a dilution of 1:4, at which dilution they were not anticomplementary. For the antigen of K. rhinoscleromatis, strain 3 obtained through the courtesy of Dr. E. Hoyt, College of Medical Evangelists, Los Angeles, was used; for the antigen of E. coli, strain 56 from our own collection was used; for the antigen of A. aerogenes, strain 884 of the American Type Culture Collection was used. In all cases the organisms were grown on beef heart agar prepared as outlined elsewhere (Rake and Oskay, 1948). All antigens were heated in a water bath at 56 C for 1 hour before use. The sera were mostly from cases of granuloma inguinale, other venereal diseases, or chronic superficial ulceration (Dunham and Rake, 1948; Rake, 1948).
The complement-fixation tests were recorded as 4+, 3+, 2+, 1+, or 0 fixation. A serum giving 2+, 3+, or 4+ fixation, with no anticomplementary activity, in a test in which all other controls were satisfactory, was considered positive.

RESULTS

Table 1 shows the results of complement-fixation tests when antigens prepared from *K. rhinoscleromatis*, *K. pneumoniae*, *E. coli*, and *A. aerogenes* are tested with human sera and the results correlated with the results obtained with *D. granulomatis* antigen and the same sera.

It will be noted that correlation of negative sera is complete. That is to say, no serum negative with *D. granulomatis* antigen gave fixation with any other of

### TABLE 1

Complement-fixation tests using antigens prepared from *K. rhinoscleromatis*, *K. pneumoniae*, *E. coli*, and *A. aerogenes*

<table>
<thead>
<tr>
<th></th>
<th><em>K. RHINOSCLEROMATIS</em></th>
<th><em>K. PNEUMONIAE</em></th>
<th><em>E. COLI</em></th>
<th><em>A. AEROGENES</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of sera tested</td>
<td>47</td>
<td>68</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>Over-all correlation with <em>D. granulomatis</em> antigen</td>
<td>94%</td>
<td>78%</td>
<td>69%</td>
<td>64%</td>
</tr>
<tr>
<td>Correlation with sera positive with <em>D. granulomatis</em> antigen</td>
<td>89%</td>
<td>63%</td>
<td>45%</td>
<td>36%</td>
</tr>
<tr>
<td>Correlation with sera negative with <em>D. granulomatis</em> antigen</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

### TABLE 2

Irregular behavior of sera in complement-fixation tests using antigens from *D. granulomatis*, *K. rhinoscleromatis*, *K. pneumoniae*, *E. coli*, and *A. aerogenes*

<table>
<thead>
<tr>
<th>SERUM NO.</th>
<th>D. GRANULOMATIS</th>
<th>K. RHINOSCLEROMATIS</th>
<th>K. PNEUMONIAE</th>
<th>E. COLI</th>
<th>A. AEROGENES</th>
</tr>
</thead>
<tbody>
<tr>
<td>118</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>165</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>182</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

the antigens tested. With sera positive to *D. granulomatis* antigen the correlation varied from 89 per cent with *K. rhinoscleromatis* antigen to 36 per cent with *A. aerogenes* antigen, suggesting a closer relationship to *D. granulomatis* to the genus *Klebsiella* than to the other two genera.

If only sera from cases of granuloma inguinale were considered, the positive correlation for *K. pneumoniae* antigen rose from 63 per cent to 74 per cent. The figures for the other antigens were not altered.

In general, sera positive with the antigen giving a lower percentage of positive correlation were positive with antigens giving a higher percentage of positive correlation. Thus sera positive with *A. aerogenes* antigen were positive with *E. coli* antigen; those positive with *E. coli* antigen were positive with *K. pneumoniae*
Antigen. In fact, only three exceptions to this rule occurred (table 2). It will be seen that in two cases there was a negative reaction with K. rhinoscleromatis antigen and in one with K. pneumoniae antigen. These negative reactions were out of line in that antigens, which in the over-all picture showed less positive correlation, were positive in these three cases.

DISCUSSION

The results of further serological tests presented here demonstrate an antigenic relationship of D. granulomatis to several members of the tribe Escherichae and support the suggestion made elsewhere (Rake, 1948) that D. granulomatis should be included in this tribe. If one accepts the degree of correlation in the positive sera as a further indication of the closeness of such relationship, D. granulomatis is closest to Klebsiella, of the three genera tested, and is particularly close to K. rhinoscleromatis (the presumed etiological agent of rhinoscleroma). The latter fact is of the greatest interest when one recalls the similarities in the etiological problems in the two diseases (Rake, 1948). Other investigators have described common antigens for K. pneumoniae and K. rhinoscleromatis (Morris and Julianelle, 1934; Gastings and Snijders, 1936). We have pointed out elsewhere the morphological similarities between D. granulomatis and K. pneumoniae as revealed by the electron microscope (Rake and Oskay, 1948).

SUMMARY

Sera fixing complement with an antigen prepared from Donovania granulomatis also gave, in a high percentage of instances, fixation with antigens prepared from other members of the tribe Escherichae, namely, Klebsiella rhinoscleromatis (89 per cent), Klebsiella pneumoniae (63 per cent), Escherichia coli (45 per cent), and Aerobacter aerogenes (36 per cent).

REFERENCES

DUNHAM, W., AND RAKE, G. 1948 Cultural and serologic studies on granuloma inguinale. Am. J. Syphilis, Gonorrhea Venereal Diseases, 32, 145-149.


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