STUDIES ON GRANULOMA INGUINALE
IV. GROWTH REQUIREMENTS OF DONOVANIA GRANULOMATIS
AND ITS RELATIONSHIP TO THE NATURAL
HABITAT OF THE ORGANISM*†

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While the postulates of Koch have never been fulfilled in granuloma inguinale, there is much presumptive evidence to indicate that Donovania granulomatis is the aetiological agent of the disease. The prototype of D. granulomatis was isolated originally by Anderson (1943), and since that time at least six other investigators (Beveridge, 1945; Dienst, 1948; Goldberg, Weaver, and Packer, 1953; Jennison, Helwig, and Milstone, 1947; Sheldon, Thebaut, Heyman, and Wall, 1945; Thomison, 1951) have isolated additional strains. The organism isolated in each of these cases was a Gram-negative pleomorphic rod which grew only in the presence of egg yolk material. Morphologically these organisms showed bipolar staining with Wright's stain and, when capsulated, closely resembled the Donovan bodies which are pathognomonic for the disease.

In addition to the morphological similarity between Donovania and the Donovan body, serological data indicate that patients with granuloma inguinale have antibodies directed against the Donovania. Anderson, DeMonbreun, and Goodpasture (1945) were able to show that an antigen prepared from one of their strains of Donovania reacted with sera from patients with the disease. Goldberg, Weaver, Packer, and Simpson (1953) have shown by complement-fixation tests that 136 out of 151 sera from patients with granuloma inguinale gave positive reactions with antigens prepared from strains of Donovania. Only one of 112 of their control sera gave a positive reaction. The data also indicated that the titres of the sera increased with the duration of the disease.

If we assume that D. granulomatis is the aetiologic agent of granuloma inguinale, two pertinent questions must be answered:

1) What is the normal habitat of the organism?
2) How is it transmitted from patient to patient?

In spite of the fact that the literature contains much reference to the venereal transmission of the disease, epidemiological evidence indicates that the disease is not spread by sexual means. For example, Packer (1959) observed that in over 500 of his cases of granuloma inguinale he was able to detect the disease in only two of the marital partners.

Recent work in our laboratory has indicated that the Donovania are not so fastidious as previously reported and that the cultural conditions necessary for growth are such that these data may shed some light on the natural habitat of the organism.

Methods

In the work reported here, four strains of Donovania granulomatis were used:

(1) The Anderson strain, isolated by Dr. K. Anderson in 1943 and obtained from Dr. A. D. Dulaney, University of Tennessee College of Medicine in 1948;
(2) The Franklin strain, isolated by the author in Memphis, Tennessee, in 1948;
(3) The Allen strain, isolated by Dr. R. B. Dienst in Augusta, Georgia, and obtained from him in 1948;
(4) The Tennessee strain, isolated by Dr. J. B. Thomison in Nashville, Tennessee, and obtained from him in 1951.

These strains were inoculated into the yolk-sac of 5-day-old embryonic eggs. After 4 days' incubation at 37°C, the yolk material was removed aseptically and inoculated in suitable amounts into in vitro egg yolk.
media previously shown to support growth of the Donovania. The Dulaney medium used is essentially 50 per cent. egg yolk in Locke's solution coagulated in the slanting position by the use of heat and then overlaid with sterile Locke's solution. The material containing viable Donovania was inoculated into the overlay.

Results

Inoculations into the in vitro medium gave contradictory results. Some growth did occur, but no consistent results were obtained. A similar situation had occurred some years previously when it was found that the diet of the hen was of great importance to the growth-promoting properties of the yolk material obtained from her eggs (Goldberg and others, 1953). Yolk material from various types of egg was substituted in the media with no better results. Finally, by a process of trial and error, it was found that the oxidation-reduction (O-R) potential of the medium was of prime importance. If Dulaney slants were prepared as usual and then overlaid with sterile fluid thioglycollate medium* instead of Locke's solution, a good growth of the Donovania would ensue, but thioglycollate medium alone or the usual infusion broths would not support continued growth of the organism. Table I shows the results obtained.

* Fluid thioglycollate medium was obtained from Baltimore Biological Laboratories, Baltimore, Maryland:

<table>
<thead>
<tr>
<th>Table I</th>
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<tr>
<td>GROWTH OF D. GRANULOMATIS IN VARIOUS MEDIA</td>
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<table>
<thead>
<tr>
<th>Medium</th>
<th>Transfer No.</th>
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<tr>
<td>Yolk + Locke's Solution</td>
<td>± 1 2 3 4 5 6 7 8 9 10 11 12</td>
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<tr>
<td>Yolk + Thioglycollate Medium</td>
<td>4 4 4 4 4 4 4 4 4 4 4 4</td>
</tr>
<tr>
<td>Thioglycollate Medium</td>
<td>3 4 ± 3 1 ± 1 1 1</td>
</tr>
<tr>
<td>Heart Infusion Broth</td>
<td>± 1 2 3 4 ± 0 0 0 0 0</td>
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= No growth. ± to 4 = Degrees of growth.

It was inferred from the results obtained that two conditions were necessary for the successful propagation of Donovania:

1. A factor or factors present in egg yolk,
2. A proper oxidation-reduction (O-R) potential.

The growth of the organism in egg-thioglycollate medium suggested that these organisms were either anaerobic or micro-aerophilic. Under suitable conditions an egg yolk medium may furnish both conditions.

After the importance of the correct O-R potential had been determined, an attempt was made to find a substance or substances which would be able to replace the factor or factors normally present in egg yolk. Numerous substances were tested but only two were found to supply the factor necessary. These substances were lactalbumin hydrolysate (an enzymatic digest of albumin supplied by Nutritional Biochemicals Corporation) and Phytone (an enzymatic digest of soya meal produced by Baltimore Biological Laboratories). When these substances were incorporated into a synthetic medium a good growth of the Donovania resulted. The medium had the following composition:

Lactalbumin hydrolysate or Phytone 20·0 gr.
Sodium chloride 2·5 gr.
Dipotassium phosphate 1·5 gr.
Sodium thioglycollate 0·6 gr.
1-cystine 0·4 gr.

The ingredients were added to the 1,000 ml. of water and dissolved with heat. The medium was allowed to cool and adjusted to pH 7·2. The medium was placed in screw-capped tubes in volumes of 20–22 ml. and sterilization was accomplished at 21°C. for 15 min. After sterilization was accomplished the caps were tightened to insure the maintenance of reduced conditions in the medium.

Interestingly enough, chemically digested pеп-tone products did not support growth of the organism.

For the past year, with over one hundred transfers, the four strains of Donovania granulomatis have been successfully cultured in the above medium.

The following characteristics of lactalbumin hydrolysate have led us to believe that the active principle is probably of a polypeptide nature:

(a) Autoclaving does not destroy the factor.
(b) Heat and 10 per cent. trichloro-acetic acid do not produce a precipitate.
(c) The factor is not dialysable through ordinary dialysing membranes.
(d) Treatment with strong acids and alkalis without heat destroys the factor.
antigen, 66 which we addition, factors inguinale, 25 of that, Anderson (1958) may share rabbit Anderson (1948)2 Franklin (1948) suggest were grown contaminated. cultured granuloma inguinale while present as = been prepared had antigens into antigens the frozen because antigens the sera K. rhinoscleromatis with granuloma inguinale. We feel that this factor is probably a peptide. These findings along with the previously published serological relationships between D. granulomatis and members of the Enterobacteriaceae, strengthen the idea of a faecal habitat of D. granulomatis.

Summary

Donovania granulomatis has hitherto been reported to grow only on material containing egg yolk material. Evidence is presented to indicate that two factors are necessary for growth:

(1) A low oxidation-reduction potential which can be satisfied by the use of a thioglycollate medium;

(2) A factor or factors found in egg for which may be substituted the enzymatic digests of bovine albumin or soya meal.

Acid hydrolysis of these materials destroys this factor. Presumptive evidence is presented to indicate that this factor is probably a peptide. These findings along with the previously published serological relationships between D. granulomatis and members of the Enterobacteriaceae, strengthen the idea of a faecal habitat of D. granulomatis.

REFERENCES

Dienst, R. B. (1948). In "Recent Advances in the Study of Veneral Diseases: A Symposium", p. 266. Venereal Diseases Education Institute, Raleigh, N.C.