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SOME STUDIES ON THE ETIOLOGY OF GRANULOMA INGUINALE*

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IN THE United States, with the exception of certain localized areas in some of the southern states, granuloma inguinale in the past has been referred to as a rare malady. Within more recent years, interest has been focused on this clinical condition primarily for three important reasons: First, the malady is becoming more prevalent and more generalized geographically; second, it is being considered by some as a fourth venereal disease; and third, there is a lack of agreement as to the nature of its etiology.

We have made microscopic examinations of nine clinical cases in Freedmen's Hospital within the last three years. Three of these patients lived within the vicinity of the District of Columbia for at least five years prior to the appearance of any lesion, and the other five came from various parts of the southern states.

Upon repeated smear examinations, seven of these nine cases showed the microscopic diagnostic picture of granuloma inguinale, when stained by the Wright or Giemsa method. The exact nature of these included bodies is not known.

Some observers agree with Donovan,¹ that it is a protozoan of the gregarine order of sporozoa; some with Flu,² that it is a chlamydozoan and there are others among whom are many of the more recent workers such as Campbell,⁶ Randall, Small and Belk⁷ who agree with Walker,³ that it is a member of the Friedländer group of bacilli.

Five of these nine cases have been reported by Poindexter.⁴ The other four cases were observed since. Three are still being treated at the Freedmen's Hospital, while one has been discharged as cured following tartar emetic and fuadin medication.

Within the last two years, attempts have been made to isolate and culture the organism in all cases that were positive by microscopic examination. Of the last four cases, three were positive microscopically. From the three positive cases of this set we were able to isolate identical organisms in two. By returning to the study of the first five cases, we were able to isolate a similar organism from one even though the patient had received considerable treatment and was improving nicely.

From the study of these three similar organisms, it is hoped that additional evidence may be shown as to the nature of the intracellular bodies (etiologic agent) in granuloma inguinale.

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The organisms which form the basis of this report were obtained from three clinical cases of the disease: Cultures from the first of these patients, M. N., one of those reported by Poindexter,⁴ show the following organisms on isolation and study: (a) A gram-negative diplobacillus; (b) a small gram-positive staphylococcus and micrococcus; (c) a short chain nonhemolytic streptococcus; (d) a diphtheroid; (4) a few large thick rods which proved to be Döderlein's bacilli; and (f) the original smear from the patient also showed a spirochete which along with a fusiform bacillus gave a picture resembling that of a Vincent's angina smear. This fusiform bacillus is believed to be the same as the Döderlein's bacillus isolated by culture.

Cultures from the area of ulceration of the second patient, J. B., showed a pure culture of an organism similar in morphology and culture to the organism (a) isolated from the first patient, M. N. Cultures from the third patient, R. F., who is now on the ward receiving treatment with fuadin, contained the following organisms: (a) A small gram-negative bacillus which resembled Friedländer's bacillus morphologically. (b) A small gram-negative coccus, which corresponded culturally to that of *Micrococcus ureae* of Cohen; the gram-negative staining character was the exception, and (c) an organism of the diphtherial group.

The organisms (a) of the first and third patient, and the pure culture from the second were selected as the organisms of special study. The fact that three similar organisms appeared in three clinical cases of granuloma inguinale and that in one of these cases a pure culture was found, is significant from an etiologic standpoint. The selection of these organisms for study is in accordance with the opinion of Walker,³ 1918, DeMonbreun and Goodpasture,⁸ 1933, and others. Their thermal death point was 51° for thirty minutes, and 59° for ten minutes. The organisms did not show capsule formation in culture. Their biochemical reactions are shown in Table I.

EXPERIMENTAL

Fourteen mice and nine guinea pigs were used for the pathogenicity test of the organism in an attempt to fulfill Koch's postulates. Five mice were inoculated intraperitoneally with a suspension of the diplobacilli isolated from the first case; three of them with a saline suspension of a twenty-four-hour agar slant culture and the other two with 1/4 c.c. of a twenty-four-hour broth culture. The mice showed no ill effects from the inoculation. After four days one of the mice was killed and cultures were made from the peritoneal cavity and the heart's blood. The peritoneal cavity showed considerable polymorphonuclear exudate and the culture was positive for the diplobacilli, but the culture from the heart's blood was negative.

Another one of the mice was sacrificed on the ninth day, with negative results. The other three mice continued to live without any ill effects for two months, after which they were used in a trypanosome experiment. Four mice were used in a similar way to test the pathogenicity of the diplobacilli isolated from the second case. The pathogenicity results were negative. Similarly five mice were used for intraperitoneal and sacrifice tests with cultures from the

third patient with similar negative results. The guinea pigs were inoculated with doses twice the size of those given the mice. Three guinea pigs were used for each organism. The guinea pigs showed no reaction for eight days, after which time they appeared to be ill. Aseptic puncture of the peritoneal cavity resulted in the removal of some exudate similar to the type observed in mice, but the cultures for the organisms were negative. Similar punctures of the heart gave negative cultures. Within the following three weeks, the animals showed progressive loss of weight which resulted in marked emaciation and death about four weeks from the date of inoculation. Cultures taken by punctures from the peritoneum and heart were negative eight days after injection and remained negative as the emaciation progressed. By repeated experiments we were able, however, to obtain positive cultures of the organisms from fluid drawn from the peritoneum within the first six days after inoculation. The stools and urine were negative for the organism. At the autopsy there were no ulcerations or areas of granulation which in any way resembled those seen in granuloma

TABLE I
THE BIOCHEMICAL REACTIONS OF THE GRAM-NEGATIVE DIPLOBACILLI OBTAINED FROM THREE CASES OF GRANULOMA INGUINALE

MEDIA	FIRST PATIENT	SECOND PATIENT	THIRD PATIENT	OTHER CHANGES IN THE CULTURES
Dextrose	a*	a	a	Turbidity and a gray yellow fine granular precipitate.
Dextrine	-	-	-	A slight surface film and precipitate.
Maltose	a	a	a	A heavy yellow precipitate and a surface film.
Saccharose	a	a	a	A gray yellow precipitate and a surface film.
Lactose	a	a	a	A stringy granular flocculation.
Salicin	a	a	± a	A thick gray film on the surface.
Mannite	a	a	a	Slight surface film and a gray yellow precipitate.
Inulin	-	-	-	Granular flocculation with a slight gray precipitate.
Inosite	-	-	-	A slight surface film and gray precipitate.
Sorbite	-	-	-	A slight surface film and slight precipitate.
Xylose	-	-	-	
Litmus milk	Sl.a*	Sl.a	a	No coagulation.
Plain agar	Grayish yellow slightly elevated. Glistening round colonies.			Similar reactions were observed for each of the other two organisms.
Peptone water	No indol formation.			Similar reactions were observed for each of the other two organisms.
Nutrient broth	Cloudy growth in 24 hours.			Similar reactions were observed for each of the other two organisms.
Gelatin	Not liquidified but bubbles of gas occur just below surface.			Similar reactions were observed for each of the other two organisms.
Nitrate reduction	±	±	+	
H ₂ S test	-	-	-	
Methyl red	±	Sl.+	+	
Voges-proskauer	-	-	±	

*a. Fermentation.

Sl.a. Slight acid without gas.

inguinale. The lung and gastrointestinal tracts grossly showed no lesions that could be considered the cause of death. Sections were not taken. Because this organism had resembled the Friedländer's bacillus in many respects, we performed comparative agglutination, absorption and precipitation tests between this organism and strains of Type II pneumococci. The results show that this organism is not similar to the Type A, B, or C of Friedländer's bacillus. However, we could not exclude it from the heterogeneous Group X of Friedländer's bacilli.

Guinea pigs and mice were also injected intraperitoneally with mixed cultures of these isolated organisms and gonococci. The results were similar to those obtained in the animals infected with the isolated organism alone.

DISCUSSION

We have observed in the nine cases which we recently studied here, and a review of many of the reported cases, that a concomitant gonorrhoeal and an occasional chancroidal infection were frequently present or were revealed in the history as existing prior to the beginning of the symptoms of granuloma inguinale. It is also noted that frequently an operation for inguinal adenitis (buboes) was performed, which did not heal.

The two cases reported by Gruhitz⁹ both gave a history of inguinal swelling which failed to heal; one after lancing and the other after pricking. A similar history is given by Shattuck, Little and Coughlin¹⁰ in a report of three cases from Boston. The extension of this unhealed incision is common in the history of granuloma inguinale cases.

The tissue transplantation en masse from one person with the disease to another susceptible person as done by McIntosh,⁵ does not permit one to draw any conclusion as to the specificity of the organisms involved, since several organisms may be present.

A survey of the reported cases shows a higher incidence among colored than white of granuloma inguinale in the United States. This is a statement of fact, the explanation of which is involved in a large number of contributory factors. Of the contributory factors, the most important in this malady as in syphilis is the greater lack of information in the nature of personal hygiene among the colored.

SUMMARY AND CONCLUSION

We have isolated identical organisms from three cases of clinical granuloma inguinale. These organisms resemble organisms of the Friedländer's group and appear similar to the inclusion bodies in cells of curettements taken from ulcerations of clinical cases of granuloma inguinale.

We believe that the "Donovan bodies" are bacteria. The organism is non-pathogenic for mice, which is a differential point from Type A or B of the Friedländer's bacillus.

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