DÖDERLEIN'S VAGINAL BACILLUS: A CONTRIBUTION TO THE STUDY OF THE *LACTO-BACILLI*.

By ROBERT CRUICKSHANK.

(*From the Bacteriology Department of the Institute of Pathology, Royal Infirmary and University, Glasgow.*)

(With 1 Text-figure.)

This study of the characters of Döderlein's bacillus is offered as a supplement to an investigation into the vaginal flora of women during pregnancy (Cruickshank and Baird, 1930). Smears and cultures were made from the vaginal swabbings of 200 pregnant women, more than half of whom were examined at regular intervals from the third month of pregnancy until term. Several cultures of Döderlein's bacillus could thus be recovered from the vagina of the same woman. Three types of bacterial flora were recognised according to the character of the direct smear—"Grade A," composed entirely of Döderlein's bacillus and representing the normal flora; "Grade B," a mixture of Döderlein's bacillus and diphtheroids; and "Grade C," consisting of a profuse bacterial mixture in which diphtheroids, enterococci and staphylococci were most numerous.

No systematic study of Döderlein's bacillus has been reported in this country and the descriptions of the organism given by German and American workers have been mostly derived from the study of a small number of strains (Jötten, 1922; Rother, 1922; Lash and Kaplan, 1926; Thomas, 1928). A review of the literature is given elsewhere (Cruickshank and Cruickshank, 1930). The difficulty of isolating Döderlein's bacillus and maintaining it in culture has been stressed as the stumbling-block to an adequate study of the organism. In the present investigation the primary isolation of the organism has not presented much difficulty, but its subsequent maintenance is not easy and many strains could not be propagated: of over 200 strains originally isolated, 60 have been studied more or less fully, but only 20 of these 60 strains are still alive after 9–18 months' sub-culture. The group of lacto-bacilli to which Döderlein's bacillus belongs forms peroxide without catalase, and like other organisms with like characters, *e.g. Pneumococcus* and *S. viridans*, is difficult to maintain on artificial culture media (see McLeod and Gordon, 1922, 1923).

Isolation.

Cultures were made within 2–3 hours after the vaginal swab was taken. The routine procedure adopted for the isolation of Döderlein's bacillus was to smear the swab over the surface of a 4-inch Petri plate containing one per cent. lactose or glucose hormone agar *plus* 5 per cent. defibrinated rabbit blood and to incubate the plate aerobically for 48 hours at 37° C. If the stained smear showed a "Grade A" flora and the swab was cleanly taken, without vulvar contamination, the result was usually a pure growth of Döderlein's bacillus. If the

https://doi.org/10.1017/S0022172400010901 Published online by Cambridge University Press

flora was mixed, Döderlein's bacillus was much less easily isolated, although if present the colonies, being smaller and less opaque, could readily be distinguished from those of diphtheroids or enterococci. Single colonies were sub-cultured on to serum agar which was used for the subsequent maintenance of the organism. At first sub-cultures were made every second day and later once a week. For the initial isolation a fermentable sugar seems to be the most essential addition to the medium; whole blood is an adjuvant and probably acts as a catalyst to destroy peroxide. Anaerobiasis is not necessary despite statements to the contrary although growth seemed sometimes to be accelerated by cultivation in an atmosphere of reduced oxygen tension. Primary culture in an acidified fluid medium is less useful than it is for the isolation of the intestinal *B. acidophilus*. Occasionally no growth of Döderlein's bacillus was obtained on lactose blood agar when incubated aerobically despite its presence in apparently pure culture in the direct smear. Frequently the first sub-culture from lactose blood agar to serum agar was unsuccessful. The addition of 0·1 per cent. glucose to the serum agar might aid growth at this stage and in subsequent sub-culture.

Morphology.

Döderlein's bacillus, like the other lacto-bacilli, is as a rule sufficiently distinctive morphologically to permit a tentative identification from a Gram-stained smear. Non-motile, non-sporing, non-capsulated Gram-positive rods of varying length and thickness and usually slightly curved, they show a lack of rigidity and have a typical grouping which differentiates them from *B. subtilis* on the one hand and diphtheroids on the other. *B. diphtheriae* in a Gram-stained film may, however, closely resemble Döderlein's bacillus. Among 60 strains studied, three main morphological types were recognised: *Type a*, most common—about 70 per cent. of the whole group—is rather slender, slightly curved and of varying length with, even in young cultures, a fair proportion of longer forms some of which tend to lose Gram's stain. The bacilli are arranged in twos and threes forming obtuse angles and Y-shaped figures, or in bundles or palisades, rather like the grouping of *B. diphtheriae* (Fig. 1). There is no chain formation, but after a few days' growth long curling bacilli become numerous and form tangled skeins. *Type b*, the second in order of frequency, is a short plump coccobacillus, either straight or slightly curved and intensely Gram-positive; there is less morphological uniformity than in the first type, a small proportion of long slender curling forms being present alongside the short thick individuals (Fig. 2). Short chains of three or four are found and clumps of the shorter forms occur. *Type c* is a streptobacillus, there being long chains of slender even-sized bacilli, and whorled masses of the organisms like those of the anthrax bacillus (Fig. 3). Only 5 out of the 60 strains were of this type. In addition, there were strains not exactly conforming to any of these three representative types, *e.g.* a short straight diphtheroid-like organism, a long thick curling type the individual members being like bits of elastic, and one unusual strain with a bulbous central

portion (Fig. 4). Notes were made of the morphological appearances of each strain when first isolated and most of them have maintained their individual

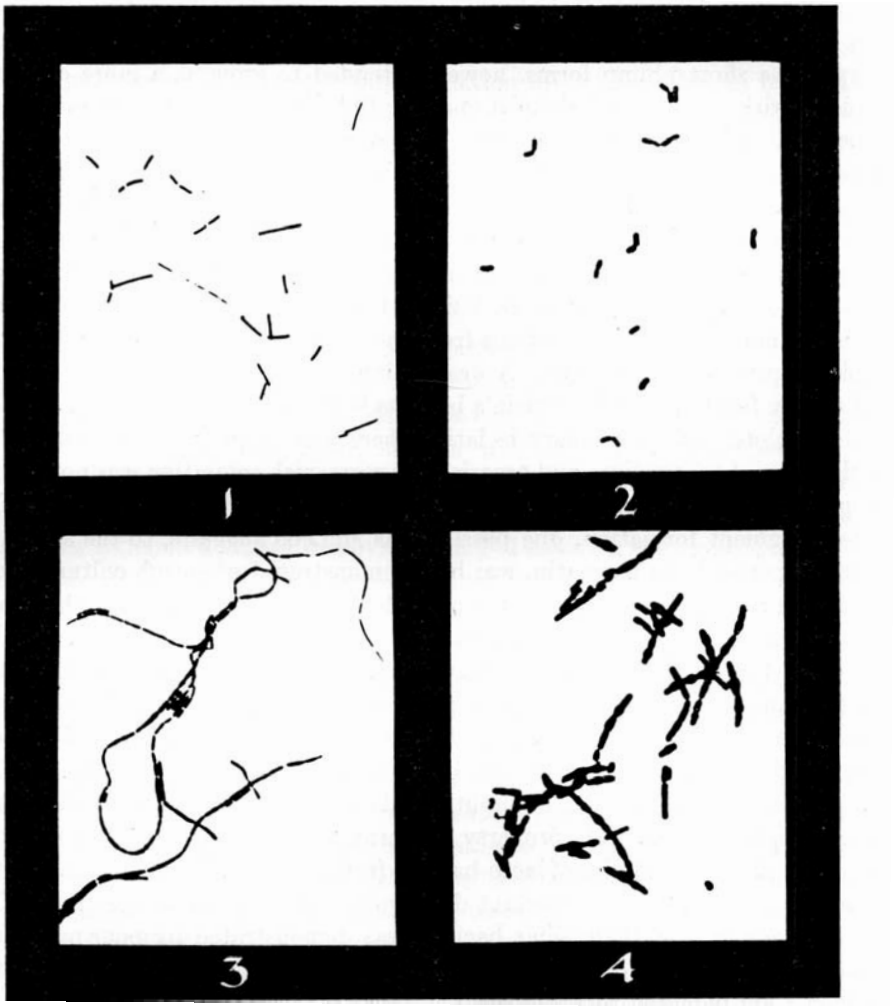

Fig. 1. *Type a*, the commonest, with grouping recalling that of *B. diphtheriae*.
Fig. 2. *Type b*, the next in frequency. Short thick individuals.
Fig. 3. *Type c*, long chains recalling those of *B. anthracis*.
Fig. 4. An unusual strain with central bulbous swelling.

characters 6–12 months after isolation. In older cultures there is an increasing pleomorphism—long curving threads, granular staining, bulbous extremities, and a tendency to lose Gram's stain.

CULTURAL CHARACTERS.

Surface colonies usually require 48–72 hours to develop fully; the growth of certain strains, even after prolonged sub-culture, is visible only after 48 hours' incubation. Individual colonies are at first very small, discrete, glistening and translucent. On the 3rd–4th day they are 1–2 mm. in diameter, slightly raised to the centre of the colony, translucent, with a matt surface

and a granular appearance by transmitted light; they have as a more characteristic feature a spreading irregularly crenated periphery. This colony appearance is similar to that of other varieties of lacto-bacilli and was common to most of the strains of Döderlein's bacillus irrespective of morphological type. The short plump forms, however, tended to grow as a more compact colony with an even well-defined margin. Colonies of diphtheroids or enterococci were bigger and more opaque. In poured plates of hormone or glucose agar, the deep colonies of Döderlein's bacillus were again characteristic of the lacto-bacillus group, the crab colony of *B. acidophilus* Y-type being a common form, *i.e.* a fairly compact colony with ragged pseudopodial outgrowths around it; others were smoother, round or lenticular, with one or two lateral buds, like the deep colony of *B. bifidus*: the delicate "feathery" colony was less common, although gradations from the "crab" to the "feathery" type of colony were frequently seen. A description (with illustrations) of the types of colony formation of Döderlein's bacillus is given by Jötten (1922).

On blood agar in primary isolation there was frequently a brownish discoloration of the medium and occasionally a greenish coloration was noticeable especially round individual colonies when these were sufficiently separate. The green-pigment formation, due possibly, as McLeod suggests, to the action of formed peroxide on haematin, was best demonstrated when sub-cultures were made on boiled blood agar—10 per cent. defibrinated rabbit blood in hormone agar heated until the medium became chocolate-coloured, and used freshly. Most of the strains were vigorous producers of peroxide, as judged by the extent and brightness of the green pigment (equivalent to *S. viridans*), some were less active, causing only a dull greenish coloration. Several strains regarded with suspicion because of their heavy growth and diphtheroid appearance were classified as diphtheroids when they failed to produce the green pigmentation. This property of peroxide production without catalase which is shared by strains of lacto-bacillus from stomach, intestines and mouth, may be regarded as an important distinguishing character of the group. The oxidative power of Döderlein's bacillus was demonstrated by pouring over a plate culture of the organism a solution of dimethyl phenylene-diamine for which I am indebted to Professor J. W. McLeod: the tetramethyl compound did not give satisfactory results (see Gordon and McLeod, 1928).

The occasional appearance of colonies of Döderlein's bacillus on MacConkey's bile salt lactose agar after plating non-catheter specimens of urine from female patients showed that the organism could grow on a medium of low-surface tension. It has been claimed that this property may help to differentiate *B. acidophilus* from *B. bulgaricus* (Albus and Holm, 1925) and explain why the former but not the latter can be implanted in the intestine. In the present study, strains from carious teeth, from stomach and intestine, as well as Döderlein's bacillus, were plated on 1 per cent. lactose agar containing 0·5 sodium taurocholate (commercial bile salt). All the strains tested grew on this medium, the teeth and stomach strains most vigorously as they do on other media. *B. bulgaricus* was not tested.

BIOCHEMICAL REACTIONS (see Table I).

Attempts have been made to subdivide the lacto-bacilli into groups according to their fermentation reactions, *e.g.* the classification of *B. acidophilus* by Rahe (1918). It has not been shown that such a grouping serves any useful purpose or helps in the differentiation of the lacto-bacilli from other organisms, although the fermentation of maltose is held to separate *B. acidophilus* from *B. bulgaricus*. All the strains of the present series were tested for their action on glucose, lactose, saccharose, maltose, mannitol, dulcitol, raffinose, salicin, inulin and litmus milk. Emulsions of a 48 hours' culture of each organism were made in sterile normal saline and the whole culture was distributed in the ten tubes each containing 5 c.c. of 1 per cent. peptone water *plus* 1 per cent. of the fermentable sugar and Andrade's indicator. Final readings were taken after 10 days' incubation at 37° C. Glucose, maltose and saccharose were almost invariably fermented, lactose less often although the litmus milk was always acidified, which may mean that milk is a better medium for growth than peptone water. Salicin and raffinose were frequently fermented, mannitol and inulin rarely and dulcitol not at all. Glucose, maltose and saccharose were fermented early—usually in 24 hours; acid production in the other fermented sugars was evident in 2–5 days. If the carbohydrate was not fermented there was no apparent growth of the organism. Litmus milk at 37° C. was acidified in 1–2 days and clotted in 3–4 days with decolorisation of the indicator from the bottom of the tube upwards. Strains of Döderlein's bacillus which were not active fermenters when first tested did not acquire any increased fermentative powers when re-tested after six months' subculture. A comparison between the fermentative reactions of Döderlein's bacillus and lacto-bacilli derived from other sources (*B. bifidus*, *B. acidophilus*, Oppler-Boas bacillus and *B. acidophilus odontolyticus*) showed that the strains from carious teeth and from gastric contents (pernicious anaemia, chronic gastritis, cancer of the stomach) are most active in the fermentation of the sugars—in particular they tend to ferment mannitol, which Döderlein's bacillus does not (see Table I).

Table I. *Fermentation-reactions of lacto-bacilli.*

Organism	Glucose	Lactose	Saccharose	Mannitol	Dulcitol	Maltose	Raffinose	Salicin	Inulin	Litmus milk
Döderlein's bacillus, 60 strains	60 (100)	42 (70)	59 (98·3)	4 (6·6)	1 (1·6)	59 (98·3)	47 (78)	44 (73)	3 (5)	A.C.D. 60 (100)
B. acidophilus (intestine), 43 strains	43 (100)	30 (70)	26 (60·4)	6 (14)	0	43 (100)	—	—	—	A.C.D 39 A. 4
B. bifidus (intestine), 9 strains	9	9	8	3	0	9	—	—	—	A.C.D. 9
B. acidophilus (stomach), 6 strains	6	6	6	6	1	6	3	4	2	A.C.D. 6
B. acidophilus odontolyticus, 35 strains	35 (100)	35 (100)	31 (88)	34 (97)	13 (37)	32 (95)	23 (67)	35 (100)	27 (80)	A.C.D. 35 (100)

The figures in parentheses denote percentages.

SEROLOGICAL REACTIONS.

Investigation was directed to finding (*a*) whether there would emerge a serological grouping of Döderlein's bacillus corresponding to the morphological

types, and (*b*) whether there exists a serological relationship between the vaginal lacto-bacillus and lacto-bacilli derived from other sources.

(*a*) Agglutinating antisera were prepared against representative strains of each of the morphological types of vaginal origin. Three strains of the most common type and one each of the cocco-bacillus and the strepto-bacillus were used, and antisera with titres of 1 : 640 to 1 : 2560 were obtained after five or six intravenous inoculations at 3-day intervals of emulsions of the living organisms into rabbits. In the performance of the agglutination tests, suspensions of an opacity equal to 500 million organisms per c.c. were prepared from 48-hour serum agar cultures, 0·4 per cent. saline being used throughout in an attempt to prevent a tendency to spontaneous agglutination. However, certain strains, particularly the cocco-bacilli, resisted all attempts to prepare a stable emulsion, *e.g.* growth in broth, repeated washing, vigorous shaking, suspension in 0·001 NaOH (see J. Smith, 1926). The agglutination tubes were incubated at 55° C. for 4 hours and read after standing at room temperature overnight.

Results. As has been mentioned, three strains of the commonest type, derived from different individuals, were used to develop antisera. Each of these strains was one of several cultures obtained at different stages of the pregnancy of the respective women. Each antiserum was therefore tested against several strains derived from the same woman, against morphologically similar strains derived from other individuals, and against strains of morphologically different type. The following results were obtained. (1) The same serological type of Döderlein's bacillus tends to persist in the vagina of an individual throughout pregnancy. Thus of four cultures derived from each of two women, all were agglutinated by an antiserum to one of the strains in each case. In the third woman, three out of four cultures were agglutinated. (2) Morphologically similar strains derived from different individuals may or may not be serologically similar. Thus 17 of 20 strains were agglutinated by one of the three antisera (quarter to full titre) whereas only 2 of these strains were agglutinated to eighth-titre by the second antiserum. The third antiserum agglutinated 9 of 12 strains tested against it. Cultures which agglutinated spontaneously are discounted. (3) Cross-agglutination does not occur with strains of morphologically different type. None of the three antisera agglutinated stable strains of the cocco-bacillary or strepto-bacillary varieties, while antisera to the two latter types failed to agglutinate 6 representative strains of the commonest type.

Of 8 cocco-bacillary strains, 5 agglutinated spontaneously: 2 stable strains were not agglutinated by the antiserum (titre 1 : 640) prepared against the remaining strain of the same type. The antiserum to the strepto-bacillus agglutinated one other similar strain, a third was unaffected and the remaining two strains clumped spontaneously in 0·4 per cent. saline.

(*b*) Regarding the serological identity of Döderlein's bacillus with other lacto-bacilli—*B. acidophilus*, *B. bifidus*, Oppler-Boas bacillus—Jötten claimed to have proved a close relationship by complement-fixation tests. On the

other hand, Lash and Kaplan failed to find any serological homogeneity in the group of lacto-bacilli by means of agglutination reactions. In the present study, antisera were prepared against *B. acidophilus* (rat intestine), *B. bifidus* and *B. acidophilus odontolyticus*. Ten strains of Döderlein's bacillus, of which two were strepto-bacilli and the others representative of the commonest type, were tested for their agglutinability by each of these three antisera. The chaining cultures were not affected but four of the other strains were agglutinated in low dilutions ($\frac{1}{16}$th–$\frac{1}{8}$th titre) by the antisera to *B. acidophilus* and *B. bifidus*. Conversely, a strain of *B. bifidus* was agglutinated to $\frac{1}{4}$th and $\frac{1}{8}$th titre respectively by two of the antisera to Döderlein's bacillus, but strains of *B. acidophilus* from stomach and teeth were not agglutinated by the same antisera. These serological relationships among the lacto-bacilli are somewhat similar to those reported by McIntosh *et alii* (1924).

SUMMARY.

It is concluded from a study of the morphological and cultural characters of 60 strains of Döderlein's vaginal bacillus that the organism belongs to the lacto-bacillus group of bacteria.

Primary culture of Döderlein's bacillus may be readily obtained on lactose blood agar incubated aerobically provided the vaginal flora is of "Grade A" type, but its subsequent propagation on artificial culture media is less likely to be successful.

Three morphological types of Döderlein's bacillus are recognised. Cultural and fermentative tests show that these types are all biologically similar.

There is no serological homogeneity among the strains of Döderlein's bacillus, although there was some evidence of a serological relationship among the members of the commonest morphological type. The same serological type tends to persist in the vagina throughout pregnancy. Döderlein's bacillus and lacto-bacilli derived from other sources are not serologically identical.

REFERENCES.

ALBUS, W. R. and HOLM, M. L. (1925). *Proc. Soc. Exp. Biol. and Med.* **22**, 337.
CRUICKSHANK, R. and BAIRD, D. (1930). *Edin. Med. J.* **37**, 135 (Proc. of Edin. Obst. Soc.).
CRUICKSHANK, J. and CRUICKSHANK, R. (1930). *System of Bacteriology*, **8**. (In press.)
GORDON, J. and MCLEOD, J. W. (1928). *J. Path. and Bact.* **31**, 185.
JÖTTEN, K. W. (1922). *Arch. f. Hyg.* **91**, 143.
LASH, A. F. and KAPLAN, B. (1926). *J. Infect. Dis.* **38**, 333.
MCINTOSH, J., JAMES, W. W. and BARLOW, P. L. (1924). *Brit. J. Exper. Path.* **6**, 175.
MCLEOD, J. W. and GORDON, J. (1922). *J. Path. and Bact.* **25**, 139.
—— —— (1923). *Ibid.* **26**, 326.
RAHE, A. H. (1918). *J. Bact.* **3**, 420.
ROTHER, W. (1922). *Centralbl. f. Bact.* I. Orig. **88**, 558.
SMITH, J. (1926). *J. Hygiene*, **25**, 165.
THOMAS, S. (1928). *J. Infect. Dis.* **43**, 218.

(*MS. received for publication* 2. II. 1931.—Ed.)