

VAGINAL CORYNEBACTERIA

BY NANCY LAUGHTON

From the Department of Bacteriology, University of Birmingham

(With Plates 5 and 6)

INTRODUCTION

In the numerous studies of Corynebacteria little attention has been paid to strains of vaginal origin. Priestley (1912), Morse (1912) and Barratt (1923) examined the biochemical reactions of a few such strains, and Tarnowski & von Büsse (1944) found five to be of the 'hyperacid' type first described by Hettche (1936).

The classification of the strains within the genus by biochemical criteria has been mainly directed to the distinction between *Corynebacterium diphtheriae* and non-pathogenic bacilli of similar morphology (Knapp, 1904; Graham-Smith, 1906; Mellon, 1917; Ebersson, 1918; Lubinski, 1921; Hompesch, 1943; Hölzl & Hauptmann, 1943).

The Medical Research Council Monograph (Andrewes, Bulloch, Douglas, Dreyer, Fildes, Ledingham & Wolf, 1923) compared the conclusions of Barratt with those of Priestley, and concluded that the biochemical grouping of strains of Corynebacteria bore no relation to their source of origin, and the opinion was expressed that the few vaginal strains which had been examined were of low fermentative power. Variation and dissociation of non-pathogenic Corynebacteria have been little studied. Morton (1940) studied variation in strains of *C. diphtheriae*. He examined a number of freshly isolated (field) strains, various subcultures of the Park 8 strain and some *gravis* and *mitis* strains received from England. He observed colonies among these which satisfied the criteria for smooth, rough and intermediate colony types. He found that the smooth colonies on nutrient agar appeared as *mitis* strains on McCleod's tellurite medium and that the *gravis* strains had the general characters of intermediate type colonies on nutrient agar. Smooth and intermediate type colonies were observed in field cultures. Smooth colonies were produced from intermediate colonies by allowing broth cultures to age or by cultivation in media containing lithium chloride. Extreme rough colonies were never isolated naturally, but were obtained in media containing lithium chloride. From these, smooth and intermediate colonies arose spontaneously. Morton suggested that the previously accepted general terms smooth and rough should have priority to the terms *mitis* and *gravis*, but he ignores the correlation of the severity of the clinical disease with the cultural type. Although some workers, notably Robinson (1934) and Carter (1946) have described dissociative and variation phenomena in strains of *C. diphtheriae intermedius*, on the whole the cultural types of McCleod appear to be fairly stable colonial variants of various substrains of *C. diphtheriae*. Their respective general characters, however, are no

doubt comparable with the smooth and intermediate colony types of other species. The terms 'S', 'SR' and 'R' have been used in this paper to describe the general features of the colonies of my collection of vaginal Corynebacteria, and have no reference to immunological or morphological characters. The work deals with 34 Corynebacteria which were derived from the vaginal flora of infertile women. They are numbered 1-38, but the strains nos. 23, 24, 33 and 35 were incompletely examined and are omitted from this paper. The organisms were assigned to the genus according to the definition given by Bergey (1939), and three control organisms were examined for comparison. Two of these were the National Collection Type cultures, *C. hofmannii*, no. 231, referred to below as *C. hofmannii*, and *C. xerosis*, no. 1042. As the latter was not considered typical a second strain, *C. xerosis* VC, isolated from the normal conjunctiva of a laboratory worker was included in the examination. These two strains contrasted strongly with each other. The strain no. 1042, which was originally isolated from a nasal source and fermented saccharose at that time, did not conform to the description of *C. xerosis* given by Bergey (1939). Its growth on nutrient agar was luxuriant and opaque. The conico-lenticular colonies were 1-1½ mms. in diameter at 48 hr. They were in the 'S' phase and resembled those of *C. hofmannii*. It reduced nitrate and constantly failed to ferment saccharose. Its colonies on Neill's medium showed variation in respect of reducing action on potassium tellurite. They were all in the 'S' phase, but some were uniformly grey and others jet black, and some of the grey colonies showed well demarcated sector-shaped areas which were jet black. This organism resembled the vaginal strains described below in subgroup B of group 3.

On the other hand, the strain of *C. xerosis* VC was much more typical. It grew sparsely on nutrient agar in small translucent colonies of only ⅓-¼ mm. in diameter. It fermented saccharose and did not reduce nitrate. Its colony on Neill's medium was small, smooth and shining with a grey-black centre and a lighter periphery. It was typical of the colonies of the vaginal strains described below in subgroup A of group 3.

METHODS

Fresh vaginal smears were examined. For primary isolation cultures were made using a range of media and incubating at 37° C. under aerobic conditions, and under conditions of reduced oxygen tension with a small amount of carbon dioxide. The morphology was recorded in direct smears and in subcultures on Loeffler's serum slopes soon after isolation. All strains were tested for motility, for haemolytic action (surface colonies on 5 % horse blood agar), for indole and hydrogen sulphide production and for their action on the following substrates, of which the fermentable substances were analytically pure: gelatin, 1 % potassium nitrate, 1 % urea, and 1 % concentrations of glucose, saccharose, maltose, dextrin, lactose, starch, glycogen, glycerol, salicin, galactose, xylose, mannite, inulin, arabinose and raffinose. In the case of the glucose-fermenting strains the rapidity and intensity of action on this substrate was noted (see Tables 1 and 2). A quantitative test was used in respect of the saccharose-fermenting strains to see whether they came into the 'hyperacid' category of Hettche and other German workers (see Table 3). In this

test the strains were grown in 5 ml. amounts of 1% saccharose broth as described by Hetteche. The actual amount of acid produced by the whole culture in 5 days was titrated with standard decinormal sodium hydroxide, and the results were recorded in ml. of that solution. Estimations were made in duplicate.

Pathogenicity was tested in two ways. Strains which fermented glucose but not saccharose were tested by the usual guinea-pig subcutaneous test for virulence in diphtheria bacilli. All strains were tested by intradermal inoculation into rabbits, using 0.2 ml. of an actively growing 24 hr. broth culture with uninoculated broth controls.

OBSERVATIONS

I have recorded elsewhere the appearance of these Corynebacteria in direct smears (Laughton, 1948*a*). My first consideration of their cultural and biochemical characters suggested a multiplicity of strains. Very few indeed were identical in every respect with one another, or even with the type species of *C. hofmannii* or *C. xerosis*. To have attempted to establish species upon minor differences would have complicated the present unsatisfactory position to an absurd degree. It was found that no help was forthcoming from the morphological or staining characters in distinguishing between groups and, also, these characters were apt to vary in the same strain even under apparently similar conditions. Analysis of the biochemical action was of limited value because there was little correlation of action on the two main substances, glucose and saccharose, and on the subsidiary substrates, such as lactose, dextrin, maltose, glycerol, nitrate or urea, one or more of which were attacked by these strains. Therefore, it was felt that grouping should rest on the broad biochemical basis of glucose and saccharose fermentation, and on cultural appearances on nutrient agar and on Neill's medium after 48 hr. incubation.

All the strains had the following negative characters: they were non-motile, non-haemolytic, they failed to liquefy gelatin, to produce hydrogen sulphide, or indole. They had no action on starch, glycogen, mannite, salicin, inulin, xylose, arabinose, or raffinose. They have been described in detail in three primary groups according to their action or lack of action on glucose and saccharose: group 1 containing the non-fermenters, group 2 containing those which fermented glucose but not saccharose, and group 3 containing those which fermented both glucose and saccharose. All the saccharose-fermenting strains also fermented glucose. Further subdivision on the basis of cultural characters was necessary among the strains of groups 1 and 3.

Group 1

Six strains, nos. 1, 13, 18, 19, 21 and 29, were placed in group 1 as they failed to ferment glucose or saccharose on primary isolation. Only one strain, no. 29, reduced nitrate and, in this respect and others, appeared to be identical with the national Type culture, *C. hofmannii*, no. 231. Differences in the cultural characters of two of the six strains necessitated a division of the group into sub-groups A and B.

Subgroup A

Four strains, nos. 1, 13, 19, 29, and *C. hofmannii*, were placed in this subgroup.

Morphologically, nos. 1, 13 and 19 were smaller than the average *corynebacterium* (see Pl. 5, fig. 1). They were short, slender rods; those of no. 13 had a pronounced curvature in the first few generations after isolation (see Pl. 5, fig. 2). No. 29 and *C. hofmannii* were stouter, short or medium-sized rods with tapered ends. All strains stained regularly Gram-positive, and all but no. 19 showed metachromatic staining by Neisser's method.

Cultural characters. The colonies of all four strains on nutrient agar resembled those of *C. hofmannii*. The growth was luxuriant and the colonies were opaque, varying in colour from whitish cream to creamy yellow, and in size from $\frac{1}{2}$ to 1 mm. They had the general characters of the 'S' phase.

On Neill's medium *C. hofmannii* grew as a medium-sized black colony with a lighter rim (see Pl. 6, fig. 5). The colony of no. 1 was similar but lighter in colour, being grey with a black centre and translucent rim. The colonies of nos. 13 and 19 were identical. They were homogeneously grey-white and larger than those of *C. hofmannii*, (see Pl. 6, fig. 4). All strains were in the 'S' phase. In nutrient broth no. 1 produced a uniform turbidity. *C. hofmannii* and the other three strains grew as a granular deposit with a clear supernatant fluid. Nos. 1, 13 and 19 neither reduced nitrates nor split urea. No. 29 reduced nitrates but was not tested for urea-splitting. *C. hofmannii* reduced nitrate and split urea.

Pathogenicity. Intradermal tests on rabbits with nos. 1, 19 and *C. hofmannii* produced localized erythematous lesions with some oedema and slight induration which resolved slowly. No. 13 gave a negative result.

Subgroup B

Two strains, nos. 18 and 21, which failed to ferment any of the carbohydrates in the series on primary isolation, differed from the strains in subgroup A in their cultural characters. Morphologically, no. 18 resembled *C. hofmannii*. It stained irregularly Gram-positive and showed metachromatic granules. No. 21 resembled nos. 1, 13 and 19 morphologically. It stained regularly Gram-positive and showed no metachromatic granules.

Cultural characters. Colonies on nutrient agar were very small, the average size being $\frac{1}{4}$ mm. Those of no. 18 were semi-transparent and some had tiny papillate centres. The colonies of no. 21 were of a similar size but were translucent and homogeneous. They had a semi-matt surface and an entire edge.

On Neill's medium the colonies of the two strains were identical and quite different from those of the strains in sub-group A. They were, in fact, characteristic of the glucose and saccharose-fermenting strains placed in group 3, subgroup A (see Pl. 6, fig. 1). In nutrient broth both strains grew as a granular deposit and no. 18 had a pellicle. Neither strain reduced nitrate. No. 21 split urea, but no. 18 did not.

Pathogenicity. When tested by intradermal inoculation in rabbits, both these strains produced erythematous lesions with oedema and slight induration.

Group 2

Five strains, nos. 5, 12, 27, 34 and 36, were placed in this group as they fermented glucose but not saccharose. Their rate and power of glucose fermentation was, however, slower and weaker than that of *C. diphtheriae* (see Table 1). Logically, the National Type strain of *C. xerosis*, no. 1042, should be placed in this category, as it failed to ferment saccharose after prolonged incubation each time it was tested. No. 12 was the only strain which reduced nitrate. Generally speaking, these strains resembled *C. hofmannii* morphologically. All but no. 27 stained regularly Gram-positive. Nos. 5, 27 and 36 showed metachromatic staining. Nos. 12 and 34 did not.

Table 1. *Glucose fermentation (strains of group 2 and C. xerosis)*

Strain	Days			
	1	2	3	5
No. 5	N/C	A	A	A/C
No. 12	S/A	A/C	A/C	A/C
No. 27	N/C	A	A/C	A/C
No. 34	S/A	A/C	A/C	A/C
No. 36	N/C	N/C	A	A/C
<i>C. xerosis</i> , no. 1042	S/A	A/C	A/C	A/C
<i>C. xerosis</i> VC	N/C	A	A/C	A/C

N/C, no change; A, acid; S/A, slight acid; A/C, acid and clot.

Cultural characters. On nutrient agar nos. 27 and 36 were very small colonies, being $\frac{1}{4}$ – $\frac{1}{2}$ mm. in diameter. Those of nos. 5 and 12 were $\frac{1}{2}$ –1 mm., and those of no. 34 were 1–2 mm. The colony of no. 27 was very flat and had a tiny papillate centre. The colonies of nos. 5, 12 and 34 showed two distinct central and peripheral components, the proportions of which varied in individual colonies of each strain (see Pl. 6, figs. 6 and 7). The periphery was typical of the 'R' phase, being semi-transparent, of a granular texture with a dry matt surface and an irregular edge. The central component, which gave the colonies a papillate or umbonate appearance depending on the relative amounts of each component, was opaque, smooth and shining. Subculture of the respective components occasionally resulted in apparently pure 'S' and 'R' variants, but on prolonged incubation at 37° C., or after a time at room temperature, the 'R' forms developed the central smooth component in the form of a small central papilla, giving an appearance of a 'daisy-headed' colony. The 'S' forms were a little more stable, and on several occasions were grown in successive generations on solid medium and their colonies looked like those of *C. hofmannii* or *C. xerosis*, no. 1042. Fluid media seemed to have the effect of causing in them a reversion to the dissociative phase, and a number of single cells isolated with a micro-manipulator (de Fonbrune), and grown overnight in fluid media, gave rise to colonies all of which were undergoing dissociation. It was, therefore, impossible to examine the fermentation reactions of the separate phases in fluid media.

On Neill's medium both 'R' and 'S' components were observed in single colonies, but the proportion of the 'S' to the 'R' component was invariably greater than on nutrient agar.

In nutrient broth all strains produced a granular growth, and nos. 5 and 12 had a pellicle also. Only one strain, no. 5, split urea.

Pathogenicity. The subcutaneous inoculation of guinea-pigs with cultures gave negative virulence tests. When tested intradermally in rabbits, two strains gave negative results and the other three produced only mild inflammatory reactions.

Group 3

The majority of strains, twenty-three in number, fermented both glucose and saccharose. The fermentation of glucose was not rapid except in the case of one strain, no. 2 (see Table 2). The amount of acid produced from saccharose quantitatively in terms of decinormal sodium hydroxide varied from 0.25 to 1.85 ml. (see Table 3). Control cultures inoculated with non-saccharose-fermenting vaginal strains, *C. hofmannii* and *C. diphtheriae*, were alkaline or gave figures below 0.2 ml. Twelve of the strains in this group gave relatively high figures. The strain of *C. xerosis* VC came into this group. It and seven vaginal strains reduced nitrate. The other sixteen failed to reduce nitrate.

Table 2. Glucose fermentation (group 3 strains)

Strain No.	Days				Strain No.	Days			
	1	2	3	5		1	2	3	5
2	AC	AC	AC	AC	17	NC	NC	NC	A
3	NC	A	AC	AC	20	NC	SA	A	A
4	NC	A	A	A	22	NC	NC	A	A
6	NC	NC	SA	A	25	NC	SA	A	A
7	A	AC	AC	AC	26	A	A	AC	AC
8	NC	NC	SA	A	28	SA	AC	AC	AC
9	NC	A	A	A	30	NC	A	AC	AC
10	A	AC	AC	AC	31	NC	NC	SA	A
11	SA	AC	AC	AC	32	SA	AC	AC	AC
14	NC	A	AC	AC	37	NC	A	A	AC
15	NC	NC	A	AC	38	A	AC	AC	AC
16	SA	A	AC	AC	<i>C. xerosis</i> VC	NC	A	AC	AC

NC, no change; A, acid; SA, slight acid; AC, acid and clot.

Table 3. Acid production from saccharose (group 3 strains)

Strain no.	NaOH (ml.)	Strain no.	NaOH (ml.)	Strain no.	NaOH (ml.)
2	0.8	11	1.525	26	0.125
3	0.625	14	1.775	28	1.4
4	1.275	15	1.575	30	0.6
6	0.625	16	1.25	31	1.55
7	1.75	17	1.5	32	1.5
8	1.075	20	1.85	37	0.25
9	0.9	22	0.725	38	0.15
10	1.3	25	1.4	<i>C. xerosis</i> VC	0.4

Subgroup A

Nineteen strains were placed in this subgroup. These were nos. 2, 3, 4, 6, 7, 8, 9, 10, 11, 14, 15, 16, 22, 25, 26, 28, 32, 37 and 38. Their morphology varied; many of them resembled *C. hofmannii* in this respect. Others were longer and more slender, resembling some forms of *C. diphtheriae* (see Pl. 5, fig. 4). One strain, no. 22, which showed marked branching in primary culture, became filamentous in subculture, but, after further subculture, it showed an ordinary diphtheroid appearance (see Pl. 5, figs. 5 and 6). With one or two exceptions these strains stained irregularly, and many bacilli in the same microscopic field failed to retain Gram's stain even in young cultures. All strains showed well-marked metachromatic staining. From this description it will be seen that a few strains resembled *C. diphtheriae* closely in their morphological and staining characteristics.

Cultural characters. Allowing for some differences in dissociative phase the strains in this subgroup gave similar colonies on nutrient agar. The majority were in the 'S' phase, being very small colonies of $\frac{1}{8}$ – $\frac{1}{2}$ mm. in diameter. They were transparent or semi-transparent, raised colonies with a smooth surface and an entire edge. Other strains were intermediate in their dissociative state and one strain, no. 25, was in the 'R' phase, and the size of its colony was somewhat bigger than the others.

On Neill's medium seventeen of the nineteen strains in this subgroup and *C. xerosis* VC. produced similar small colonies in the 'S' phase (see Pl. 6, fig. 1). Some of them were mucoid in appearance (see Pl. 6, fig. 2). The colonies were grey-black and most of them had a lighter peripheral rim, giving an appearance of a unit of frogs' spawn. The surface was smooth and shining and the edge entire. One strain, no. 3, threw 'SR' variants. The eighteenth strain, no. 22, gave colonies of an intermediate phase which closely resembled the colonies of *C. diphtheriae intermedius* (see Pl. 6, fig. 3). The nineteenth strain, no. 25, gave colonies in the 'R' phase which were similar to, but smaller than, the 'daisy-headed' colonies of *C. diphtheriae gravis*.

The growth in nutrient broth of all these strains varied with the dissociative phase of the organisms, from one of uniform turbidity to a granular deposit with pellicle formation, but, as in the case of group 2 strains, fluid media favoured the 'S' to 'R' direction of change. *C. xerosis* VC grew as a granular deposit. Ten strains split urea; the remaining nine failed to do so. No correlation between this power and nitrate reduction was seen.

Subgroup B. Four strains of group 3 differed from those in subgroup A in their cultural appearance on nutrient agar and on Neill's medium. They were nos. 17, 20, 30 and 31. Morphologically, they resembled *C. hofmannii*. All but no. 20 stained regularly by Gram's stain and all showed metachromatic staining.

Cultural characters. On nutrient agar three strains produced conico-lenticular, opaque, cream-coloured colonies with a smooth, shining surface and entire edge. They were very definitely bigger than the colonies of the strains in subgroup A, being 1–2 mm. in diameter. The general appearance of the colonies was similar to those of *C. hofmannii* and *C. xerosis*, no. 1042. The fourth strain, no. 30, had a

similar colony but one approaching the 'R' phase. It was semi-opaque, had a matt granular surface, an irregular edge and a papillate centre.

The colonies of the four strains on Neill's medium were not identical. They were large, low conical colonies varying in colour from light-grey to black. Of the three in the 'S' phase no. 17 was dark grey with a lighter rim, no. 20 was uniformly black, and no. 31 was light-grey with a black centre and a narrow sago-like rim. No. 30 in the 'R' phase was uniformly black. The colony of *C. xerosis*, no. 1042, on Neill's medium has already been described above. In nutrient broth no. 30 produced a granular deposit and a pellicle. The other strains produced either a uniform turbidity or a stringy deposit. *C. xerosis*, no. 1042, produced a uniform turbidity. Nos. 20 and 30 failed to split urea or reduce nitrates. No. 17 split urea but failed to reduce nitrates. No. 31 reduced nitrate but failed to split urea.

Pathogenicity of strains in group 3. In the case of members of each subgroup the reaction obtained in the intradermal tests on rabbits varied from one of marked inflammation to no reaction. For the sake of comparison with *Corynebacteria* studied by other workers the biochemical reactions of the strains in groups 2 and 3 are shown in detail in Table 4.

Table 4. Biochemical reactions (groups 2 and 3)

Substrate	Strain no.														
	2	3	4	5	6	7	8	9	10	11	12	14	15	16	17
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saccharose	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+
Maltose	+	+	+	+	+	+	±	±	-	+	+	±	+	+	-
Dextrin	-	±	±	±	±	±	±	-	±	±	-	±	-	±	-
Glycerol	-	-	-	+	-	-	±	-	-	-	+	-	-	-	-
Lactose	-	-	-	-	-	-	-	-	±	-	±	-	-	-	-
Galactose	-	-	-	±	-	-	-	-	-	-	-	-	-	-	-
Urea	+	-	+	+	-	+	-	-	+	+	-	+	-	-	+
Nitrate	+	+	+	-	+	-	-	+	+	+	+	+	-	-	-

Substrate	Strain no.														
	20	22	25	26	27	28	30	31	32	34	36	37	38	X VC	X 1042
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saccharose	+	+	+	+	-	+	+	+	+	-	-	+	+	+	-
Maltose	±	+	+	+	+	+	+	±	+	±	±	-	+	-	-
Dextrin	±	+	-	-	-	+	-	-	+	±	±	-	-	-	-
Glycerol	-	±	-	-	+	-	±	-	-	±	±	-	-	-	-
Lactose	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Galactose	-	±	±	-	-	-	-	-	+	-	-	-	-	-	+
Urea	-	-	+	+	-	+	-	-	+	-	-	-	-	-	-
Nitrate	-	-	+	+	-	+	-	+	+	-	-	-	+	-	+

- , negative; + , positive; ± , weak positive. (After prolonged incubation.)

COMMENTARY

If allowance is made for variability within the species, the characters of the strains of group 1, subgroup A conform to those of *C. hofmannii*. The two strains of group 1, subgroup B, however, appear to be different. Their colonies on nutrient agar

were distinct and their colonies on Neill's medium were typical of the carbohydrate-fermenting strains of group 3, subgroup A. One showed evidence of dissociation and descendent cultures of this strain subsequently acquired weak glucose-fermenting properties. It seems probable that these strains were, in fact, degraded carbohydrate-fermenters whose rightful place should be in groups 2 or 3.

One obvious importance of some of the strains within group 2 lies in a superficial resemblance to *C. diphtheriae*. They do not appear to be identical strains, although nos. 5 and 12 resembled each other quite closely. All fermented maltose and glycerol like *C. diphtheriae*, but they differed in their action on dextrin, lactose, galactose, urea and nitrate. The relationship of the members of this group to named species is doubtful. Some of them may have been variants of saccharose-fermenting strains, or avirulent forms of *C. diphtheriae*.

Making due allowance for the different cultural phases, the strains of group 3, subgroup A, appear to belong to one species. The appearance of the smooth colonies on nutrient agar was similar to that of *C. xerosis* VC and the colonies on Neill's medium were quite typical for all the members of this subgroup which were in the 'S' phase, and for *C. xerosis* VC. Examples of all cultural phases were seen in broth cultures. Variation in individual strains was seen in nitrate reduction, in urea-splitting, in action on the subsidiary carbohydrates, and in the quantitative production of acid from saccharose which showed a wide range of variation. Those strains with high values must correspond with the 'hyperacid' strains described by Hettche and other German workers. On the other hand, strains such as nos. 37 and 38 gave low values similar to that of *C. xerosis* VC. Evidently this character is very variable in members of this species. It is possible that the hyperacid strains play a definite role in the production of the high vaginal acidity normally found in the adult woman before the menopause as *Corynebacteria* appear to be commonly present, and even more frequent than Döderlein's vaginal bacillus. This view has been expressed elsewhere by the author (Laughton, 1948*b*).

The strains of group 3, subgroup B, would appear to belong to a different species. Their more luxuriant and opaque growth on nutrient agar seems similar to that of the coccoid form of *C. enzymicum* described by Mellon (1917), an organism which possessed a wide range of fermentative power and appeared to be the subject of dissociation, and his description of the cultural appearance of the bacillary form of that organism corresponds to that of the vaginal strains of subgroup A described above.

The colonies on Neill's medium of the strains of subgroup B were also quite different from the typical colonies of the strains of subgroup A, but they were not all identical with one another. That of no. 20 would fit in with the description 'large black colony', stated by Acton (1946) to be the usual form of *C. xerosis* on Neill's medium and, in fact, it resembled the jet black colony of *C. xerosis*, no. 1042. All four strains came into the hyperacid category, but their action on the subsidiary substrates was varied. On the whole, they might be considered to belong to a single species, but their exact relationship to the species which have received the specific status of *C. xerosis* and *C. enzymicum* is not clear.

These vaginal strains are remarkable for their instability; strain variants are

apparent in all groups and subgroups. Some of their cultural types are analogous to those of *C. diphtheriae*, and the stability of even these has been questioned by Menton (1932) and Christisen (1933). As stated above, Robinson (1934), and Carter (1946), have given instances of dissociative phenomena in *C. diphtheriae* which are similar to those shown by some of these vaginal strains of Corynebacteria. It is possible that some of the strains placed in group 2 are avirulent diphtheria bacilli, but this would have to be confirmed serologically. Ordinarily, the difference between avirulent diphtheria bacilli and certain diphtheroid bacilli is hypothetical because specific sera are not commonly used to distinguish such strains.

The question whether some strains of Corynebacteria other than *C. diphtheriae* have pathogenic properties is an important one. Hölzl & Hauptmann (1943) have demonstrated the presence of diphtheroid bacilli of the hyperacid type in inflammatory conditions of the middle ear, the mastoid air cells, and the accessory nasal sinuses. Strong, in a personal communication, states that the finding of diphtheroid bacilli in mastoid wounds is invariably associated with delayed healing. The inflammatory reactions produced in rabbits by many of these vaginal strains show that they are definite irritants and may be pathogenic in some circumstances. It is especially likely that they are capable of aiding other agents of vaginal, aural, or wound infection.

SUMMARY

1. Thirty-four vaginal Corynebacteria have been grouped and subgrouped according to their cultural and biochemical characters. Owing to their instability and to the rigid taxonomy of the Corynebacteria, the exact identity of many of the strains is uncertain, but their characters have been compared with some of the Corynebacteria which have been given specific rank.

2. The majority of strains were carbohydrate-fermenters and many were of the hyperacid type. These may be associated in some way with a normal high vaginal acidity.

3. Many instances of resemblances to *C. diphtheriae* in morphology, in staining reactions, and in cultural appearances relating to the different dissociative phases, were encountered in the examination of these strains, and, in fact, some of the cultural types seen were analogous to those of *C. diphtheriae* described by McLeod.

4. Intradermal tests on rabbits showed that many of these strains, including some from each group, were capable of inciting inflammatory reactions in their tissues, and it is suggested that they may play a secondary part in some infections.

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EXPLANATION OF PLATES

PLATE 5

- Fig. 1. Strain no. 1 (group 1). × 800. Small, slender bacilli.
 Fig. 2. Strain no. 13 (group 1). × 800. Curved forms.
 Fig. 3. Strain no. 28 (group 3). × 800. Showing coccoid and bacillary forms.
 Fig. 4. Strain no. 6 (group 3). × 800. Long, slender, irregularly staining bacilli.
 Fig. 5. Strain no. 22 (group 3). × 1600. Branched forms.
 Fig. 6. Strain no. 22 (group 3). × 800. Filamentous forms undergoing fragmentation.

PLATE 6

- Fig. 1. Strain no. 10 (group 3). × 16. 'S' phase.
 Fig. 2. Strain no. 8 (group 3). × 16. 'M' phase.
 Fig. 3. Strain no. 22 (group 3). × 16. 'SR' phase.
 Fig. 4. Strain no. 13 (group 1). × 16. 'S' phase.
 Fig. 5. *C. hofmannii*, no. 231. × 16. 'S' phase.
 Fig. 6. Strain no. 34 (group 2). × 16. Showing dissociation.
 Fig. 7. Strain no. 12 (group 2). × 20. Showing central and peripheral components.

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