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THE CULTIVATION AND BIOLOGICAL CHARACTERS OF BACILLUS ACNES

BY

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AND

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THE CULTIVATION AND BIOLOGICAL CHARACTERS OF BACILLUS ACNES.[1]

By H. J. Südmersen, Ph.D.; and E. T. Thompson.

From the Wellcome Physiological Research Laboratories, Herne Hill, S.E.

(Plate VII.)

In the year 1894 Unna and Hodara observed and described for the first time the existence of a bacillus in the comedones of true acne, but were unable to obtain it in culture. Later, in 1897, Sabouraud described the organism as being the causative agent of acne and alopecia areata. He was the first to cultivate the organism with some success and to draw attention to the extreme variability in form, size, and disposition.

No further advance appears to have been made towards the definite establishment of its relationship to the disease until the work which has recently appeared by Fleming, in which he supports his contention by results obtained by him on the opsonic indices of acne patients, the beneficial effects of treatment by a vaccine prepared from the bacillus, and by a successful inoculation experiment performed on a susceptible subject by means of a pure culture of the organism. In this paper the author describes two different media on which he succeeded in cultivating the organism,—the one, the most suitable, being prepared from ordinary agar neutralised with HCl to which glycerin and oleic acid is added; the other, ordinary agar slightly acidulated with HCl.

On account of its simplicity we determined to try whether a good culture medium could be obtained by acidulation only, and the success in this direction justifies, we believe, the publication of our results. A series of preliminary trials made for the purpose of determining the proper degree of acidity were first made on ordinary agar. This was found to be equal to + 40 phenol-phthalein. The addition of various substances including lanolin, brain, and blood was next tried, with the result that by far the best growth was obtained on acid blood or serum agar medium in the proportion of one part horse blood[2] or one part serum added to three parts of a 3 per cent. nutrient agar made + 40 acid to phenol-phthalein. The blood or serum is run aseptically into agar kept liquid at a temperature not exceeding 60° C., the whole is uniformally mixed and allowed to solidify. On this medium an appreciable growth is obtained in less than twenty-four hours from material directly obtained from an acne lesion; this increases and becomes very thick on the second day. After repeated subcultivations an abundant growth appears after an interval of twenty-

[1] Received June 8, 1909.

[2] Probably blood from other sources would be as efficient.

Fig. 1.

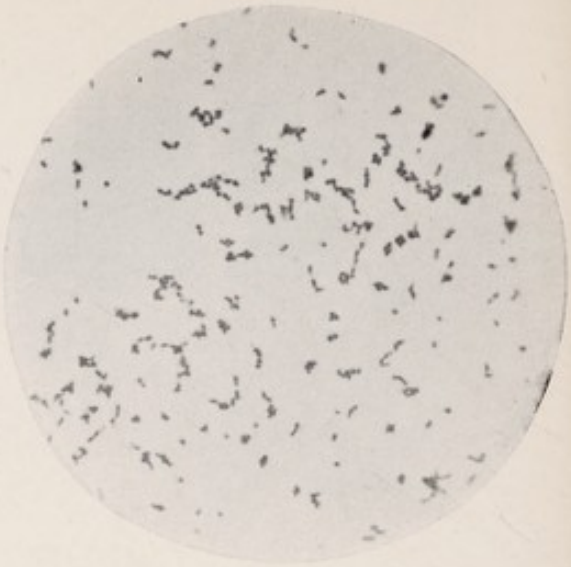

Fig. 2.

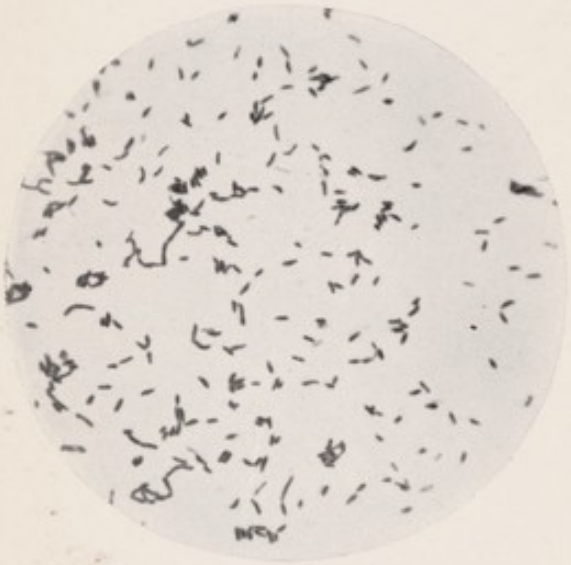

Fig. 3.

Fig. 4.

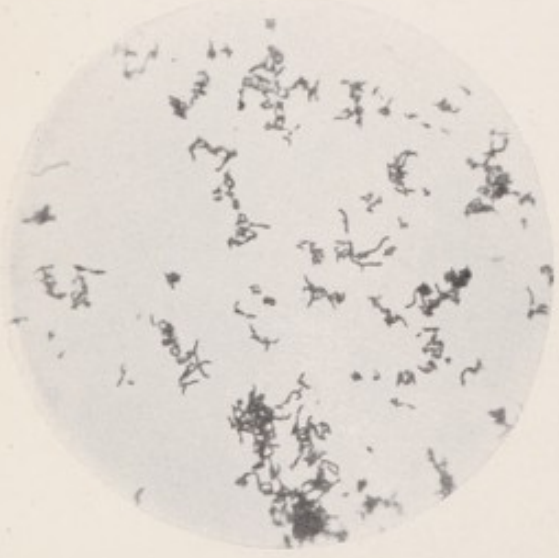

Fig 5.

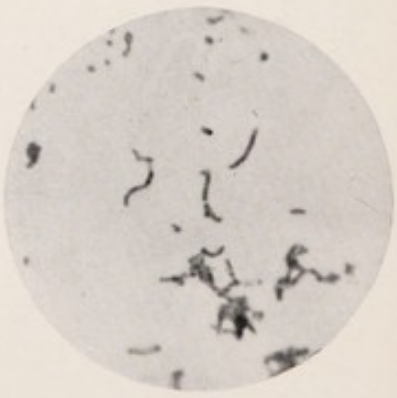

Fig. 6.

four hours on acid serum agar forming a dull grey opaque layer, the organisms possessing a long filamentous and branched character; isolated colonies are greyish white, non-adhesive, and readily removed *en masse* from the surface of the medium by the platinum needle.

One of the strains (Strain I.) used in our investigation was kindly supplied by Dr. Spitta of St. George's Hospital. Strain II. was isolated from an assistant of the laboratory staff by the following method. The affected part of the skin was thoroughly cleansed with a weak lysol solution and dried by means of alcohol, then by pressure the contents of the pustule were squeezed into the bore of a sterilised watch key and transferred to a sterile Petri dish, the deeper portion was cut off and broken up by rubbing between two sterile glass slides with the addition of a little sterile saline solution to form a uniform suspension from which material was taken to inoculate the surface of acid serum agar. A pure culture was easily obtained, the colonies of staphylococcus being few in number. When first isolated, growth takes place best anaerobically, but after several generations on artificial media an equally good growth is obtained aerobically. We have by this means always succeeded in cultivating the organism from material taken from the deeper layers of the comedo where the conditions must be strictly anaerobic.

Cultural Characters.

On *acid serum agar* the organism maintains its vitality under the best conditions for about four weeks, after which time it soon dies; its optimum temperature of growth is 37° C.; no growth takes place at a temperature of 22° C.

The following media were inoculated from vigorously growing cultures on acid serum agar of Strain I. and II. and incubated for seven days:—

Ordinary neutral agar.—All attempts to adapt it to growth on this medium were unsuccessful.

Glucose agar stab.—Aerobic.—Dense growth extending to within a $\frac{1}{4}$ inch of the surface. *Anaerobic* growth takes place along the entire track to the surface.

Ordinary neutral broth.—Aerobic and *anaerobic.*—A greyish granular deposit growth; the broth remains clear.

Neutral red broth.—Aerobic and *anaerobic.*—Growth good; no change occurred.

Loeffler's medium, malachite green agar, Endo agar.—Aerobic and *anaerobic.*—No growth.

Litmus milk.—Aerobic and *anaerobic.*—Growth slight; no change.

These results show that an acid medium is not necessary for the cultivation of the organism; slope glucose agar may be employed, but strictly anaerobic conditions appear then to be essential. We have been unsuccessful in our attempts to adapt it to aerobic growth on this medium, although inoculations were made from cultures which grew readily on the same medium anaerobically and on acid serum agar aerobically and anaerobically equally well.

Fermentation of various carbohydrates, etc.—The table shows the results of the tests carried out on the two strains. Since growth takes place as a rule very slowly, marked alterations in reaction are usually not shown until after the expiration of several days. The observations were continued up to the fourth week of incubation. It will be noticed that there are slight differences exhibited by the two strains both as to time of appearance and degree of reaction, Strain II. being the more active. Gas is in no case produced.

In the preparation of the media used for the following tests 1 per cent. of the substance is added to a mixture consisting of 25 parts ordinary neutral

1 per cent. peptone broth and 75 parts, 1 per cent. peptone water; litmus solution is added and neutralisation effected by addition of dilute HCl.

TABLE.

		Glucose.	Galactose.	Saccharose.	Lactose.	Maltose.	Raffinose.	Starch.	Inulin.	Arabinose.	Amygdalin.	Arbutin.	Salicin.	Glycerin.	Erythrol.	Dulcite.	Mannite.
Strain I.	Aerobic . .	+	+	±	0	+	0	...	+	+	0	0	...	+	0	0	+
	Anaerobic .	+	+	±	0	+	0	...	+	+	0	0	...	+	0	0	+
Strain II.	Aerobic . .	+	+	+	0	+	+	+	+	+	0	0	...	+	0	0	+
	Anaerobic .	+	+	+	0	+	+	+	+	+	0	0	...	+	0	0	+

+ = acid; ± = slightly acid; 0 = no reaction.

Glucose.—Aerobic and anaerobic cultures of both strains give rise to strong acid reaction. In Strain I. the acidity does not appear until after the second or even third week, while Strain II. becomes strongly acid at the end of the first week. An abundant growth occurs.

Galactose.—Results throughout the same as with glucose.

Saccharose.—A difference is shown in the degree of acidity in the two strains. Strain I. has but little effect, a faint acid reaction is first shown after fourteen days; this becomes but slightly stronger in three to four weeks. Strain II. produces a markedly acid reaction at the end of the first week. Growth is but slight.

Lactose.—Moderate growth.

Maltose.—In this medium Strain I. is much less active than Strain II. The latter produces a marked acidity at the end of the first week, the former not until the second or third week of incubation.

Raffinose.—This constitutes a differential medium for the two strains; Strain I. causes no change either in aerobic or anaerobic culture. Strain II., on the contrary, produces a marked acid reaction. Both organisms grow well.

Starch.—Strain I. could not be induced to grow in this medium. Strain II. gave rise to a slight degree of acidity in three weeks, which became marked at the end of four weeks. The acidity was stronger and appeared earlier aerobically.

Inulin.—Strain I. gave slight acid at the end of fourteen days in both aerobic and anaerobic culture; in the latter bleaching occurred in twenty-one days. Strain II. gave stronger acidity without bleaching.

Arabinose.—Both strains gave marked acidity in aerobic and anaerobic culture. This was shown after seven days, being somewhat stronger in Strain II. Growth is moderate.

Amygdalin.—Appears to be unaffected; growth is slight.

Arbutin.—Was unaffected; growth slight.

Salicin.—Neither strain appeared to grow in this medium.

Glycerin.—A strong acid reaction appeared in Strain II. within seven days, in Strain I. not until the end of the second week. The reaction occurs earlier in aerobic culture. Growth is moderate.

Erythrol and *Dulcite.*—Slight growth.

Mannite.—Strain II. produces a strong acid reaction sooner than Strain I. The reaction is more marked and appears earlier in aerobic culture. Growth is abundant.

The most marked differences in the above are exhibited in the indifference of Strain I. to raffinose, and in its much weaker action on saccharose as compared with Strain II.

Morphology and Biology.

In a smear prepared from the contents of a pustule the organism appears as short diphtheroid forms, one end being often enlarged; small coccoid and club-shaped forms are present. It stains uniformly by Gram's method and readily with all the aniline dyes, and is not acid-fast. When grown anaerobically on acid serum-agar it assumes a short coccoid appearance similar to a micrococcus. This is well shown in Plate VII. Fig. 2, whereas in aerobic growth, on the same medium, long filamentous forms which exhibit true branching predominate. This remarkable difference in its appearance is shown in Plate VII. Figs. 1 and 2. When anaerobic subcultivations of the aerobic-grown long branching forms are made on acid serum agar the short coccus forms reappear.

In glucose-agar stab the organism has the diphtheroid form seen in smear preparations from pustules; pear-shaped and spindle forms are associated with slightly curved or straight forms with rounded or truncated ends (Plate VII. Fig. 3).

Plate VII. Fig. 4 shows sickle forms on blood agar culture under aerobic conditions.

Marked segmentation and beaded structure is shown when culture is made anaerobically on potato (Plate VII. Fig. 6).

A tendency to form clumps is shown in acid broth, the organism is diphtheroid; club and dumb-bell shaped forms are present (Plate VII. Fig. 5).

Thermal death point.—Sabouraud states that a high resistance to heat is possessed by this organism, prolonged heating at a temperature of 65° to 67° not being sufficient to destroy its vitality. We have been unable to confirm this with our strains, both of which are destroyed at a temperature of 60° C. maintained for thirty minutes. The tests were performed by using an emulsion in ordinary neutral broth of a forty-eight-hour culture on acid serum agar.

Relationship to B. diphtheriæ.—In its general appearance (Plate VII. Fig. 5), branching and various involution forms, and in several of its fermentation reactions, a relationship to *B. diphtheriæ* appears to be indicated. A comparison of its reactions was made with those of two strains of the *B. diphtheriæ*. *B. acnes* is generally more active in the production of acid; gas is produced by neither.

Glucose, galactose, and starch are fermented by both organisms; while lactose, amygdalin, erythrol, and dulcite are unaffected. *B. diphtheriæ* also agrees with Strain I. in not fermenting raffinose.

On the other hand, differences appear in the following:—Saccharose, inulin, and mannite are unaffected, while maltose, arabinose, salicin, and glycerin are but slightly affected by *B. diphtheriæ*.

Pathogenicity.

A 6-day culture of Strain II. on acid blood agar was used.

Rabbit 1 (weight, 690 grms.).—Injected intravenously with two loopfuls showed no symptoms. Its weight fell to 674 grms. four days later, after which it steadily rose to 815 grms. fourteen days later.

Guinea-pig F (weight, 300 grms.).—Received two loops intraperitoneally, four days later its weight had dropped to 277 grms.; after this a rise occurred. The animal was killed by the administration of $CHCl_3$ fourteen days after injection, its weight being 325 grms. Examination revealed the presence of a few nodules in the spleen, slight enlargement of some mesenteric glands, and a few areas of consolidation in the lungs.

Guinea-pig D (weight, 315 grms.).—Two loops were injected subcutaneously. After a drop to 305 grms., which took place two days later, the weight rose rapidly to 345 grms. on the sixth day. The condition remained good throughout.

Mouse C (weight, 22 grms.).—Received one loop intraperitoneally. Its weight fell to 19 grms. Death occurred ten days after injection.

Mouse B (weight, 24 grms.).—One loop subcutaneously. Death took place two days after injection.

Four loopfuls from the surface of a 5-day culture on acid serum agar were injected into each of two guinea-pigs,—in the one case subcutaneously, in the other intraperitoneally. A fall in weight occurred, but both animals survived without apparent ill effects.

Two mice were injected each with two loopfuls of the same culture, the one subcutaneously and the other intraperitoneally. Death occurred on the second and third day respectively.

The results given above show that *B. acnes* is not fatal to guinea-pigs when injected either intraperitoneally or subcutaneously; on the other hand, mice are susceptible and death results from either method of administration.

Post-mortem examinations of the mice were made; the following appearances were presented: *Spleen*—Enlarged to more than twice the normal size. *Mesenteric glands*—Enlarged and congested. *Liver*—Soft. *Kidney*—Enlarged, soft, anæmic. *Suprarenals*—Congested. *Lungs*—Partially consolidated in varying degree. *Inguinal and axillary glands*—Enlarged and congested.

The acne organism was recovered in pure culture from the seat of subcutaneous injection, and its presence was demonstrated microscopically in the spleen pulp. No growth, however, was obtained on media inoculated with the peritoneal fluid, or portions of mesenteric glands.

Filtrates from broth cultures one month old when injected either subcutaneously or intraperitoneally into mice even in doses of 1·5 c.c. produced no noticeable effects.

Conclusions.

1. A simple medium favourable to the growth of *B. acnes* is easily prepared by the addition of 1 part of horse serum to three parts of a 3 per cent. nutrient agar previously made + 40 acid to phenol-phthalein.

2. The direct cultivation of the organism from the lesion is uniformly successful under anaerobic conditions. After a few generations of anaerobic cultivation on acid serum agar growth may be obtained equally well aerobically.

3. The existence of at least two distinct strains of *B. acnes* has been demonstrated. Strain I. has no action on raffinose and very slight action on saccharose, while Strain II. acts strongly on both. Other minor differences in the degree and time of reaction are shown.

4. Remarkable morphological changes are exhibited according to the medium used for its cultivation.

5. A probable relationship to *B. diphtheriæ* is shown in many of its characters.

6. The organism is fatal to mice, but non-fatal to guinea-pigs.

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DESCRIPTION OF PLATE VII.

Fig. 1.—Film from a 3-day acid serum agar culture under aerobic conditions, showing filamentous branching forms. Gram stain. (×1000 diameters.)

Fig. 2.—Film from a 3-day acid serum agar culture under anaerobic conditions, showing coccoid forms. Gram stain. (×1000 diameters.)

Fig. 3.—Film from a 14-day stab glucose agar culture, showing diphtheroid forms. Gram stain. (×1000 diameters.)

Fig. 4.—Film from a 7-day blood agar culture under aerobic conditions, showing sickle forms. Gram stain. (× 1000 diameters.)

Fig. 5.—Film from a 7-day acid broth culture, showing clumped diphtheroid forms. Gram stain. (×1000 diameters.)

Fig. 6.—Film from a 14-day culture on potato, under anaerobic conditions, showing segmented forms. Gram stain. (×1000 diameters.)

Gram-negative areas in the above micro-photographs appear less dark than the Gram-positive.