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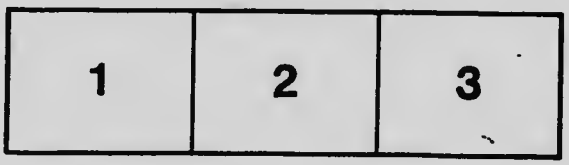

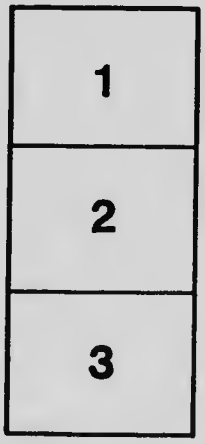

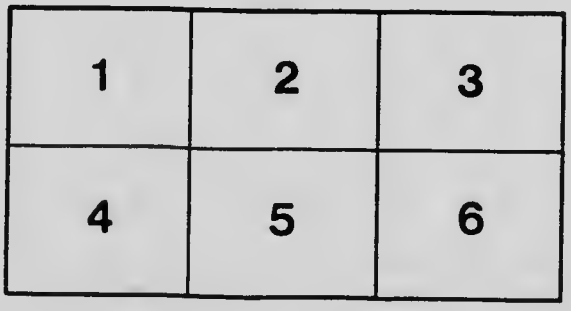

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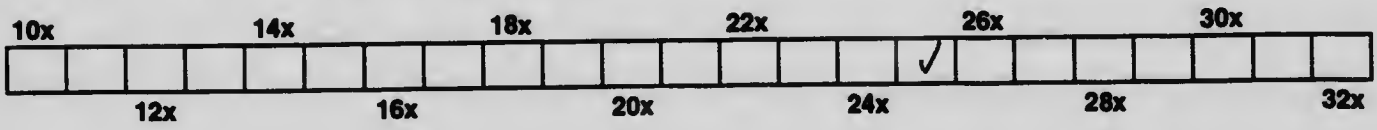

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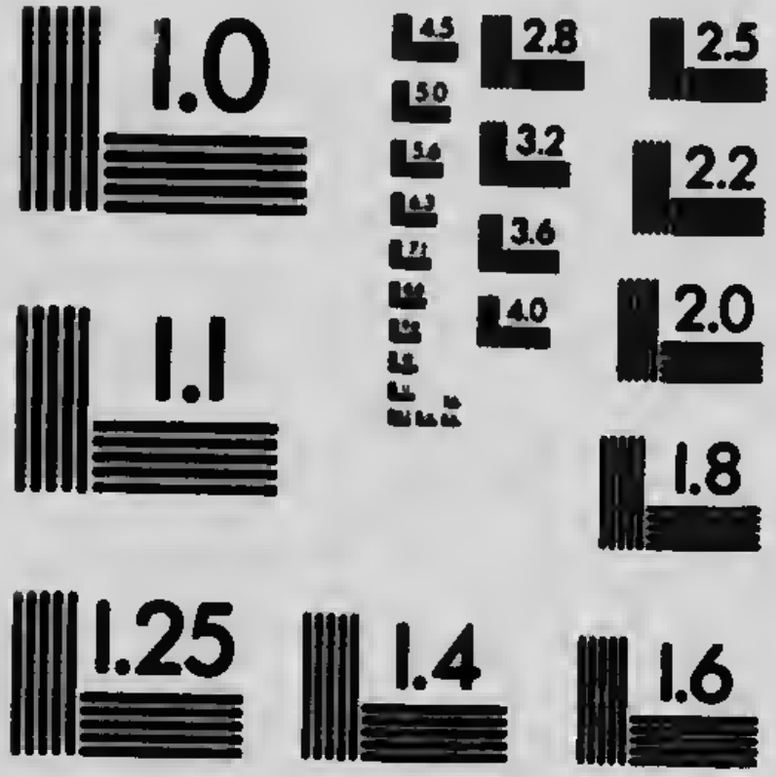

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8 GEORGE V SESSIONAL PAPER No. 38a A. 1918

BACTERIAL DESTRUCTION OF COPEPODS

OCCURRING IN MARINE PLANKTON.

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XIII.

BACTERIAL DESTRUCTION OF COPEPODS OCCURRING IN MARINE PLANKTON.

During the summer of 1916 I was investigating the bacteriological content of "Swelled Canned Fish" for the Biological Board of Canada at the Marine Station, St. Andrews, N.B.

While there Dr. Arthur Willey (Professor of Zoology, McGill University) called my attention to the condition of some of the copepods—(*Calanus finmarchicus*—upon which he was conducting researches. Under the microscope it was seen that many parts of the tissue of copepods which had died in culture flasks were completely destroyed by masses of what appeared to be bacteria. It was particularly noticed that the axial cavity in the first antennae was entirely occupied by a dense column of writhing organisms. Tubes of nutrient broth were inoculated direct from the copepods and after two days' incubation at room temperature a definite clouding of the medium was noted.

At the request and on the suggestion of Dr. Willey I have proceeded with the examination of the cultures secured, and have obtained in pure culture the organisms concerned. Three specific strains of bacteria have been isolated.

Inasmuch as the work may have some practical significance in relation to the general subject of marine biology, and is of scientific interest, this report of the detailed studies of these organisms has been prepared.

MEDIA EMPLOYED.

I began by using various media prepared from fish concoctions in addition to the ordinary laboratory media. The latter, however, proved to be more satisfactory in every way and I have therefore confined myself to their use entirely.

Beef Peptone Agar.—Standard methods[1]—Beef extract being substituted for meat.

Beef Peptone Gelatine.—Standard methods.[1]

Glucose Agar.—1% glucose added to agar prepared as above, immediately before tubing.

Sodium Indigo Sulphate Agar.—3 per cent. sodium indigo sulphate with per cent. glucose added to neutral agar, tubed and sterilized in flowing steam for three successive days.

Tochtermann's Serum Agar.—[2] For digestion test.

Löeffler's Blood Serum.—[3] " " "

Aesculin Agar.[4]—For specific reaction of organisms of the colon-aerogenes group. Loops of a broth culture spread on plates.

Neutral Red Bile Salt Agar.[5]—Ditto, ditto.

Bouillon for Voges-Proskauer reaction.[6]—

Bouillon for the Methyl Red Reaction.[7]—

Solution for reduction of Nitrates to Nitrites.—Giltay's synthetic solution was used, and also a peptone potassium-nitrate solution.

Dunham Solution for Indol Production.—1 per cent peptone, 5 per cent NaCl dissolved in distilled water, the reaction adjusted to + 10, medium cleared with white of egg, filtered, tubed and sterilized. After 7 days' incubation at 37½°C. the cultures were tested for indol by the Bohme Ehrlich test[8]; the development of a cherry red colour indicating the presence of indol.

Fermentation broths.—The various sugars, alcohols, glucosides used were prepared separately as 10 per cent solutions in distilled water, and sterilized for 15 minutes in flowing steam for three successive days. Immediately before inoculation these were added to tubes of broth made up as for the indol test—the use of peptone water without beef eliminates any risk of the reaction being masked by action on the muscle sugar—in such proportions as to give a final 1 per cent sugar or other carbohydrate broth. Dunham tubes were used for the collection of the gas. For acid production the acid fuchsin indicator of Andrade,[9] as adapted by Hollman, was used at the rate of 2 per cent.

In the preparation of the indicator I have noticed as reported by Andrade, and Hollman that the colour which results from the addition of the normal caustic soda is perceptibly affected by being left open to the air. By adding the caustic soda to freshly prepared acid fuchsin solution at intervals throughout the day, leaving the reagent meanwhile exposed to the air, I have found that 2½ cc. n/NaOH will decolorize to the proper shade of amber 100 cc. fuchsin solution.

Litmus Milk.—The milk freshly separated and tubed was sterilized for three successive days for 30 minutes in flowing steam. The litmus was made up separately; a 7 per cent solution of "Merck's" litmus in distilled water, heated in the steamer for 30 minutes and left over night in the incubator, filtered, sterilized for three successive days in flowing steam and added to the milk immediately before inoculation at the rate of 1½ per cent.

NOTE: It will be seen from page 224 that culture III of this paper exhibited an unusual degree of sensitiveness to the litmus. For this reason I now consider the proportion of the indicator added to be of some importance

CULTURAL STUDIES.

Culture I.

Morphology.—Microscopically—24-hour-old agar culture at 37°C.—short rods varying up to 1-6 μ long and 1 μ broad; some larger forms; stains unevenly with Kuhne's methylene blue, and is Gram negative. No spores are formed and no capsule shown.

Motility.—Decided brownian movement, but not the violent agitation noted in culture III. No motility.

Cultural Characteristics:—

Agar Slope.—24 hours at 37°C. growth luxuriant, raised, slightly spreading, moist, glistening, porcelain-white, edges echinulate.

Gluc. gar Slope.—Gas, growth luxuriant, raised, moist, glistening, woolly appearance, haze, porcelain-white, spreading.

Tochtermann's Serum Agar Slope.—Resembling growth on glucose agar, but no woolly appearance. In 8 days growth had permeated medium as flakes; gas, heavy precipitate collected at base of slope.

Löeffler's Blood Serum.—Moderate, spreading, flat, no digestion, no discolouration. In 7 days no digestion; colour isabella, luxuriant, moist, slightly raised, iridiscent.

Sodium Indigo Sulphate Agar Slope.—Luxuriant, raised, moist, spreading, no reduction. In 8 days no reduction.

Gelatine Stab.—21°C. 24 hours, growth filiform, equal surface and stab. In 7 days as before; gas bubbles—presumably from the muscle sugar in the beef extract—in tube. In 6 weeks no liquefaction, growth brown, echinulate, medium unchanged.

Nutrient Broth.—37°C. 24 hours. Clouding abundant, medium clear, flaky sediment at bottom, bluish rim at top. In 3 days flocculent yellowish-white rim at top, easily dislodged on shaking. Medium almost clear.

Potato.—Abundant along track of needle, glistening, contoured, isabelle colour, growth slightly raised; in 3 days iridiscence perceptible and medium slightly browned.

Milk.—Congulation in 24 to 30 hours; curd broken by gas bubbles. In 6 weeks curd contracted, no digestion

Litmus Milk.—In 20 hours lilac, much gas, no coagulation; in 36 hours congulation with gassy curd; in 5 days curd bleached. In 6 weeks no digestion.

Aesculin Agar.—Luxuriant, moist, black reaction

Neutral Red Bile Salt Agar.—Luxuriant, raised, glistening, moist. Characteristic red reaction.

Peptone Broth + Aesculin.—Black reaction.

Gelatine Colonies.—(1st appearance) 5 days at 21°C. Surface colonies up to 1 mm. diameter, raised, slightly darker in centre, paling towards edges. Under the low power objective homogenous, granular, edges entire.

Agar Colonies.—24 hours at 37°C. Surface colonies up to 3 mm. diameter, raised, concave, glistening, yellowish-white at centre, paling towards edges, edges entire, colonies bluish by transmitted light. Under low power objective edges entire, finely granular, amorphous.

Temperature Relations:—

Thermal Death Point.—10 mns. exposure in nutrient broth at 60°C.

Optimum Temperature.—37°C. Cultures incubated at 37°, 21°, and 14°C. respectively.

Vitality on Culture Media ...etive cultures have been recovered from agar after 5 months at temperatu... 15°-20°C.

Relation to Oxygen.—Facultative anaerobe; glucose agar.

Biochemical reactions:—

Indol production: Indol produced.
Reduction of nitrates. Nitrates reduced to nitrites.
Voges-Proskauer reaction: Negative.
Methyl red reaction: Acid.

Fermentation of Carbohydrates:—

Glucose.	Lactose.	Saccharose.	Maltose.	Mannite.	Dulcite.
+ +	+ +	+	+ +	+ +	+ +
Dextrine.	Salicin.	Raffinose.	Adonite.	Inulin.	Xylose.
+ +	+ +	+ +	+ +	+ +	+ +
Glycerine.					
+ +					

\+ = acid.
++ = acid and gas.

Culturally and biochemically this organism is a variation of the *B. coli* type according to the description of Escherich.[10] The variety I have isolated differs from the original description in that it is non-motile and ferments saccharose to acid and gas. The degree of importance to be attached to any one character has been discussed at considerable length in the literature during the last thirty years; owing to the fact that this organism is used as a presumptive test for faecal contamination in systematic water analysis. Of the two variations from the original type mentioned above, the presence or absence of motility may first be considered.

There has been a tendency by some workers to consider a non-motile form of *B. coli* (Escherich)[10] as *B. aerogenes* (Escherich)[11]. This position, however, is not substantiated by the researches of Escherich and Pfaundler, MacConkey, Jackson and others. Escherich and Pfaundler[12] in describing the original *B. coli* state that generally there is motility, sometimes slight; a characteristic movement as of short forward pushes; swinging in space with sometimes no change of place is also noted. The absence of definite motion as recorded by Tafel, Frankel and others is cited in the same paper. Lembke[13] considers that motility in *B. coli* is variable. McWeeney[14] in discussing what he would regard as the genuine *B. coli* remarks: "on the motility of individuals or its absence I hesitate to lay much stress." Houston[15] in using a broad classification for the true colon group adopts his "flaginae" test which leaves open the question of motility. Durham[16] considers that all members of the true colon group are probably motile; but in the same paper states: "speaking generally morphological characters are not of much value for subdivision of these bacteria."

MacConkey[17] discusses the influence of temperature and medium on motility; and while he considers the presence or absence as important he says: "it is very difficult to arrive at a conclusion with regard to this character." Ellis[18] has proved the presence of flagella in five species of the genus *Bacterium* which were hitherto held to be non-motile; and he considers that all the genus *Bacterium* when suitably cultivated can be shown to be motile. His conclusions would appear to be not sufficiently substantiated on the data given. The English Commission on the Standardization of Methods for the bacteriological examination of water[19]; and the American Commission on Standard Methods[1] each specify motility as one characteristic of the true *B. coli*; but a comparison of the two standards reveals variance as to the significance to be attached to this specific feature. Prescott and Winslow[20] consider the sugar fermentations, particularly the fermentations of glucose and lactose, are of prime importance. Savage[21] considers motility as one of the essential characters of the true *B. coli*. Migula[22] includes *B. neapolitanus* (Emmerich)[23] which is non-motile, as identical with *B. coli* (Escherich).

Thus while the concensus of opinion is undoubtedly in favour of specifying motility as a character of the true *B. coli*, there would seem to be no justification according to present classification for excluding from this type an organism preponderatingly similar and placing it with *B. aerogenes* (Escherich)[11] on account solely of the absence of motility. Harrison[28] raises the question as to whether, provided the argument *re* motility is admitted, it removes *B. neapolitanus* to a different genus from *B. coli*.

The second variation to which I have referred (page 219) is the fermentation of saccharose to acid and gas. *B. coli* (Escherich)[10] has no action upon saccharose. Theobald Smith, cited by Prescott and Winslow[20] stated in 1893 that *B. coli* could be divided into two distinct sub-types,—the one negative to saccharose or in other words the original *B. coli*, and the other fermenting this sugar to acid and gas. Durham[16] isolated saccharose—positive organisms and gave the name *B. coli communior*, since contracted to *B. communior*. Jackson[24] has classified the organisms of the lactose fermenting type and confirms the sub-type *B. communior* of Durham. The classification of Jackson has since been adopted by the laboratory section of the American Public Health Association,[1] and on this continent has received almost general approval. Using saccharose and dulcite as differential fermentation tests Jackson considers

those organisms positive to lactose and dulcite as *B. coli* (Escherich)[10]; positive to lactose, saccharose and dulcite as *B. communior* (Durham)[16]; positive to lactose and saccharose but negative to dulcite as *B. ærogenes* (Escherich)[11], positive to lactose but negative to saccharose and dulcite as *B. acidi-lactici*.[24] Further subdivision according to the action on mannite and raffinose are used for further differentiation.

MacConkey uses the Voges-Proskauer reaction as one of his differential tests and finds that the true *B. coli* is always Voges-Proskauer negative, while the *B. ærogenes* type is Voges-Proskauer positive. In the same paper he revives the name *B. neapolitanus* (Emmerich)[23] and uses this nomenclature for his saccharose positive dulcite positive strains instead of the name given by Durham—*B. communior*. MacConkey obtained a pure culture labelled *B. neapolitanus* from Kral, and out of 480 coli-like organisms isolated from human and animal fæces he found that 23 per cent gave biochemical reactions identical with the Kral culture used by him as control. He states that he cannot agree with Migula in describing *B. neapolitanus* (Emmerich) as identical with *B. coli* (Escherich). As, however, the differentiation by means of carbohydrates other than glucose and lactose has been amplified since the classification by Migula, the conclusions of both Migula and MacConkey on this particular point are perfectly legitimate. Jordan[25], in designating the saccharose-positive dulcite-positive group uses *B. communior* and *B. neapolitanus* interchangeably; biochemically this as correct, but the former is motile (16), the latter non-motile[23]. Levine[26] who apparently follows MacConkey has lately studied 333 strains of lactose fermenting bacteria from various sources. He goes one step further and giving *B. neopolitanus* its original character of non-motility according to Emmerich[23], uses that nomenclature to include non-motile forms of *B. communior* (Durham). To say the least it is interesting to revive *B. neapolitanus* as a sub-type of *B. coli* (Escherich) in view of the following statement by Jordan[25a]: "According to a strict application of the rules of priority, the bacillus now known as *B. coli* should be called *B. neapolitanus*." The dates of the original publication by Emmerich [23a], and Escherich[10], of course bear out Jordan's statement.

However, according to the first descriptions of Emmerich[23] and Escherich[10] the former found a non-motile strain and the latter a motile strain of a lactose fermenting organism. Later work already referred to has separated these two strains on the basis of saccharose fermentation[28]. We thus have two features in which the respective strains differ. A propos of the stand taken by Durham and McConkey, Harrison[28] opens the question as to whether it is legitimate to name as a species, an organism differing only in the fermenting of one sugar.

It would therefore seem legitimate, on the ground of present day classification, to tentatively characterize the organism I have isolated-a non-motile, lactose, saccharose, dulcite positive, Voges-Proskauer negative strain,—as a variety of the sub-type *B. neapolitanus* of the classic *B. coli* type of Escherich. To use *B. neapolitanus* conflicts with the nomenclature *B. communior* more usually accepted for the strains giving identical reactions. If motility is considered, *B. neapolitanus* and *B. communior* are not strictly the same; but to use the single characteristic, absence or presence of motility, to separate *B. communior* and *B. neapolitanus*, and at the same time to say that a non-motile form of colon is identical with a motile form may seem inconsistent.

The difficulty can be overcome by the tentative classification of the organism I have isolated as non-motile strain of the sub-type *B. communior* (Durham) of the type *B. coli* (Escherich); or to take the differentation further, as *B. neapolitanus*, a sub-type of *B. coli* (Escherich).

Culture II.

Morphology.—Microscopically—24-hours-old agar culture at 37°C.—rods varying up to 1·6 μ long and ·8 μ broad; some not much longer than broad; stains evenly with Kühne's methylene blue and is Gram negative. No spores; no capsules have been demonstrated.

Motility.—Rapid movement, darting to and fro, many revolve as on an axis.

Cultural Characteristics:

Agar Slope.—24 hours at 37°C.—moderate, bluish by transmitted light, moist, glistening, slightly raised, later becoming by transmitted light yellowish in centre gradually merging into transparency.

Glucose Agar Slope.—Gas, growth moderate to luxuriant, glistening, slightly raised.

Tochtermann's Serum Agar Slope.—Moist, slightly raised, bluish by transmitted light, spreading discrete colonies, gas. In 8 days growth had become yellow, much water of condensation, heavy greyish-white precipitate at base of slope.

Löeffler's Blood Serum.—Moderate, filiform, moist, glistening, no liquefaction, no discolouration. In 7 days no digestion, no discolouration.

Sodium Sulphate Agar Slope.—Raised, spreading. moist, no reduction. In 8 days no reduction.

Gelatine Stab.—21°C., 24 hours, growth filiform, equal surface and stab; 7 days, tendency to echinulate. In 6 weeks no liquefaction, growth yellowish-brown; characteristic lateral growths resembling a poplar tree against the horizon; medium unchanged.

Nutrient Broth.—37°C. 24 hours. Clouding abundant, no pellicle, no sediment, bluish rim at top. In 1 week, slight sediment; otherwise no change.

Potato.—Moderate, flat, yellowish-white along track of needle.

Milk.—In 6 weeks no change.

Litmus Milk.—Varies from no change to a tint slightly more alkaline than control; blue rim at top.

Aesculin Agar.—Black reaction, growth less luxuriant than in Culture I.

Neutral Red Bilesalt Agar. Moderate, pink reaction.

Peptone Broth + Aesculin.—Black reaction.

Gelatine Colonies.—5 days at 21°C.—colonies up to 5 mm. diameter; under low power objective granular; edges lobular to contoured, centre dark with paling towards edges. Deep surface colonies granular centre with dark concentric rings.

Agar Colonies.—24 hours at 37°C.—surface 1 mm. diameter, raised, concave, bluish by transmitted light, round, smooth, edges entire. Under low power objective granular, edges entire.

Temperature Relations:—

Thermal death point: 10 minutes exposure in nutrient broth at 55°C.

Optimum temperature: 37°C.; cultures incubated at 37°C., 21°C. and 14°C. respectively.

Vitality on Culture Media:—

Active cultures have been recovered from agar tubes after 5 months at temperature of 15°-20°C.

Relation to Oxygen:—

Facultative anaerobe; glucose agar.

Biochemical reactions:—

Indol production: Indol not produced.
Reduction of nitrates: Nitrates reduced to nitrites.
Voges-Proskauer reaction: Positive, after 6 hours.
Methyl red reaction: Faint acidity, shortly followed by reversion to alkalinity.

Fermentation of Carbohydrates:—

Glucose.	Lactose.	Saccharose.	Raffinose.	Maltose.
++	± –	++	– –	++
Mannite.	**Dulcite.**	**Adonit.**	**Salicin.**	**Dextrine.**	**Inulin.**
++	– –	– –	++	++	– –

Xylose. ++ Glycerine. ++ (slowly).

\+ = acid.

++ = acid and gas.

NOTE.—The fermentation of lactose to acid is faint, and in two days reduction is noted.

The classification of this culture must be purely tentative. It will be seen that while saccharose, maltose, mannite, salicin and dextrin are fermented to acid and gas, the organism fails to ferment lactose to gas and only faintly to acid. This has persistently been the case through several months; on one occasion, however, a small bubble of gas—1 mm. diameter—appeared in a Durham tube. This I have been unable to obtain since, confirming in triplicate. MacConkey states: "It has been my experience that where an organism produces acid and gas in one medium and apparently only acid in another, under proper subcultivation the organism will produce gas in the second medium."[17] Harrison in this laboratory has frequently cited to me verbally his own experience in this matter, which bears out the statement of MacConkey. While the organism is definitely motile it differs from *B. cloacae* of Jordan[29] in that it fails after three months to liquefy gelatine, fails to ferment lactose to gas, and fails to coagulate milk after several weeks. Rogers Clarke and Evans[30] found that the group of the types they isolated from grains—Group B—fermented to acid and gas glucose, saccharose, mannite, glycerine and adonit, but like my culture failed to ferment lactose; on the other hand this group liquefied gelatine.[30] These workers consider that such group has at best only a slight connection with the *colon-ærogenes* group. Taking the classification adopted by the American Public Health Association[1] the culture would be ruled out of the *colon-ærogenes* group at once on account of its failure to produce gas from lactose; further, milk is not coagulated. Certain of the biochemical reactions would tend to suggest the *Gaertner* group. According to Besson[31] the organisms of this group are negative to lactose, saccharose, salicin, raffinose and inulin; while those carbohydrates to which the group is positive include dulcite. This organism, it will be noted, is negative to dulcite, lactose and inulin but positive to saccharose and salicin. Jordan[32] in a study of 74 strains of the *Gaertner* group cites that the reaction to dulcite and xylose is variable, but includes dextrine among the fermentable substances not attacked; thus establishing at once a similarity and a variation respectively as compared with the organism here described. In the same paper Jordan describes strains where reaction to litmus milk cannot be differentiated from the control. Savage[33] in a classification of the *Gaertner* group divides such into two sub-groups:—

a. *True-Gaertner bacilli;*
b. *Para-Gaertner bacilli;*

to which he had previously drawn attention in reports to the Local Government Board, 1906-7-8. Citing from Savage: "The bacilli of the para-Gaertner sub-group are a number of organisms, for the most part unnamed, which appear to be not very uncommon in the healthy animal and human intestine, and which are of chief interest from their close resemblance to *true-Gaertner* bacilli. . . . They can only be culturally differentiated from the *true-Gaertner* organisms by an extended series of fermentation tests while they fail to be agglutinited by immunizing animals with

any of the members of the *true-Gaertner* sub-group. They are also for the most part non-pathogenic. They have not so far been found as a cause of disease in man or in animals."

Until I am able to secure for comparative cultural tests strains of this sub-group from Dr. Savage, it would not be wise to attempt a more definite classification of the organism herein discussed. In view, however, of the decided variation from the Voges-Proskauer type of the *colon-ærogenes* group as lately given by Levine,[29] and considering the many cultural features and fermentative reactions which suggest at any rate a distant relationship to the *para-Gaertner* group, it seems not undesirable to suggest that based on the cultural features and biochemical reactions this organism be considered tentatively as an atypical form of the *para-Gaertner* group according to Savage.[33]

Culture III.

Morphology.—Microscopically the organism appears as a coccus, in pairs, in masses, and as short streptococci; the average diameter from a 24-hour-old agar culture at 37° C. being .8 μ, stained with Kühne's methylene blue. The organism is Gram positive and non-spore-forming; capsules faintly discernible.

Motility.—Tests for motility made in hanging drop of condensation water from a young agar culture. No motility. Violent agitation can be noticed, and rotation of the cells as on an axis, but the position in the drop is unchanged.

Cultural Characteristics:—

Agar Slope.—24 hours at 37° C. growth scanty, bluish by transmitted light, filiform, flat, with later a tendency to spreading.

Glucose Agar Slope.—Growth moderate, heavier than on agar, discrete colonies, flat, spreading, glistening.

Tochtermann's Serum Agar Slope.—Growth scant to moderate, bluish by transmitted light, heavy clouding of the condensation water. In 5 days slight digestion of the medium noted.

Löeffler's Blood Serum.—Growth filiform, medium channelled and slightly darker in colour. In 5 days growth glistening, yellowish, slight digestion.

Sodium Indigo Sulphate Agar Slope.—Faint growth, no reduction of colour, 24 hours. In 14 days reduced to reddish brown.

Gelatine Stab.—21° C. In two days liquefaction beginning. In 7 days stratiform liquefaction for ⅓ of tube, even clouding with yellowish flocculent precipitate at bottom. Liquefaction complete in 1 month.

Nutrient Broth.—37° C. even clouding, moderate, no pellicle, no sediment; later medium cleared.

Potato.—Barely discernible growth in 24 hours. In 3 days faint growth, flat, spreading, white, metallic lustre.

Milk.—37° C. In 36 hours weak coagulum, no gas noted. In 72 hours digestion had begun, a clear lemon coloured liquid extending for ⅓ tube. In 7 days tube half fluid, curd soft, gelatinous, bright and of a solidity resembling macaroni; easily desintegrated on shaking; after 2 months some curd still remaining, lemon yellow in colour, consistency as before.

Litmus milk.—The reaction of the organism to this medium is unusual, and it is due to the sensitiveness here discovered that I have adopted the uniform percentage of litmus, noted on page 218. If litmus be added at the rate of 1½ per cent coagulation preceded by bleaching takes place within 36 to 48 hours. Digestion then begins and proceeds slightly more rapidly than in the milk, the contents of the tube varying in colour from a lemon yellow to claret with decided fluorescence in 72 hours. In 2 months digestion is not complete, 1-2 cm. of a jelly-like claret coloured curd remaining.

If the quantity of litmus added be more than 1½ per cent the reaction is quite different, varying according to the percentage of litmus added. There may or may not be coagulation, the colour varying from isabella to a muddy purpureus; flakes of tinted curd can later be noted. In 2 months a condition resembling broken jelly of a variety of shades of purpureus has been recorded. A note referring to this phenomenon in greater detail is being published elsewhere.

Aesculin agar.—Growth moderate, flat, dry, brown to black.

Neutral Red Bile Salt Agar.—Growth scant, no characteristic colour reaction.

Peptone Broth Aesculin.—Black in 12 hours.

Gelatine Colonies—(1st appearance).—21°C. 4 days, punctiform to pinhead colonies, depression in medium commencing; under the low power objective structure compact, finely granular, paler towards the edges; edges ciliate.

Agar Colonies.—37° C. growth slow. 24 hours colonies .5 mm. in diameter, growth tends to be subsurface. Under the low power objective colonies round or eliptical, edges entire to undulate, internal structure granular, dark halo in surrounding medium.

Temperature Relations.—

Thermal death point. 10 minutes' exposure in nutrient broth at 60°C.

Optimum temperature. 37°C.; cultures incubated at 37°C., 21°C. and 14°C. respectively.

Vitality of Culture Media:—

Active cultures have been recovered from agar tubes after 5 months at temperature of 15°-20°C.

Relation to Oxygen.

Facultative anaerobe. Under anaerobic condition on glucose agar, growth visible in 24 hrs. at 37°C.

Biochemical Reactions:—

Indol production: no indol in 7 days.
Reduction of nitrates: no reduction to nitrites.
Voges-Proskauer reaction: negative.
Methyl red reaction: acid to methyl red.

Fermentation of Carbohydrates:—

Glucose.	Lactose.	Saccharose.	Maltose.	Mannite.	Duleite.
+	+	+	+	+	– –
Dextrin.	**Salicin.**	**Raffinose.**	**Adonite.**	**Inulin.**	**Xylose.**
+	+	– –	– –	– –	– –
Glycerine.					
– –					

\+ = acid.
++ = acid and gas.

In accordance with the cultural results this organism is properly included among the liquefying streptococci. Winslow[34] takes the *Str. gracilis* of Escherich, Lehmann and Neumann as the "type centre" of these liquefiers. He considers that the various streptococci which peptonise gelatine more or less actively are variants of this type; intermediate between it and some of those characterized by Andrews and Horder[35].

I find, however, a closer resemblance to an organism described by MacCallum and Hastings[36] as *Micrococcus zymogenes.* This was isolated from a fatal case of acute endocarditis, and while it shows the same main characteristics as *Str. gravilis,* in

liquefies serum slightly and subsequent to coagulating milk digests the clot. This organism was later found by Birge.[37] It is in the two last characteristics that I find the close resemblance to *M. zymogenes* noted above. The original description of *Str. gracilis* of Escherich cited by Winslow[34] includes non-liquefaction of blood serum and failure to coagulate milk; but summing up the variations Winslow provisionally defines his "type centre" *Str. gracilis* as follows: Small coccus, appearing in chains, ferments lactose and coagulates milk, may ferment mannite and salicin, liquefies gelatine actively.

While the organism I have described appears to have certain particular characteristics, I hesitate to depart from Winslow's view regarding the relationship of the variants in his tentative group of streptococcus liquefiers [34]. I conclude therefore that this organism which culturally and biochemically is identical with the *M. zymogenes* of MacCallum and Hastings[36] should be placed as a variety of the type *Str. gracilis*.

SUMMARY AND CONCLUSIONS.

1. Three strains of bacteria have been isolated from the destroyed tissue of copels which had died in culture flasks.

2. Summarized, the biological features are as follows:—

—	I. Rod-form.	II. Rod-form.	III. Coccus.
Gram's Stain	−	−	+
Spores	−	−	−
Capsule	−	−	+
Motility	−	+	−
Agar	Luxuriant	Moderate	Scant.
Gelatine	No liquef.	No liquef.	Liquef.
Potato	Abundant	Moderate	Scant.
Loeffler's Blood Serum	No digestion	No digestion	Slight digest.
Milk	Coagulag	No change	Coag. and digest
Thermal death pt.	60°C.	55°C.	60°C.
Optimum temperature	37°C.	37°C.	37°C.

3. Summarized, the biochemical reactions are:—

—	I.	II.	III.
Indol	+	−	−
Nitrate reduction	+	+	−
Voges-Proskauer	−	+	−
Methyl Red	Acid.	Faintly acid, later alkal.	Acid.
Glucose	++	++	+
Lactose	+−	±−	+
Saccharose	++	++	+
Raffinose	++	−−	−−
Maltose	++	++	+
Mannite	++	++	+
Lulcite	++	−−	−−
Adonite	++	−−	−−
Salicin	++	++	+
Dextrine	++	++	+
Inulin	+	−−	−−
Xylose	++	++	−−
Glycerine	++	++	−−

+ = acid. ++ = acid and gas.

4. Based on their cultural features and biochemical reactions the organisms are classified as follows:—

Culture I.—Tentatively as a non-motile strain of the sub-type *B. communior* (Durham) of the type *B. coli* (Escherich); or to take the differentiation further, as *B. neapolitanus*, a sub-type of *B. coli* (Escherich).

Culture II.—Considered tentatively as an atypical form of the *Para-Gaertner* group after Savage.

Culture III.—Identical with *M. zymogenes* and placed as a variety of the type of liquefying streptococci, *Streptococcus gracilis.*

5. No inoculations of these cultures have been made into healthy copepods owing to distance from the sea.

6. It is not legitimate to draw any definite conclusions regarding the relationship of these organisms to the destruction of the copepods, as no inoculation experiments have been carried out, and the postulates of Koch have not yet been satisfied. According to the descriptions presented, however, the evidence is strong in favour of Culture III being a possible causal agent.

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REFERENCES.

1. American Public Health Association, 1915—"Standard Methods Water Analysis," 77-137.

2. Besson, 1913—"Text Book Practical Bact., etc.," (Longmans), 53.

3. Besson, 1913—"Text Book Practical Bact., etc.," (Longmans), 52.

4. Harrison and Vanderleck, 1908—"Aesculin Bile Salt Agar for Water and Milk Analysis," Trans. Roy. Soc. Can. III. Ser. II, 105-110.

5. Savage, 190 '—"Bacteriological Examination of Water Supplies," London, 221.

6. Voges and Proskauer, 1898—"Zeit. fur Hyg." 28, 20.

6. Harden, 1905, 1906—"On the Voges-Proskauer Reaction for Certain Bacteria," Proc. Roy. Soc., 77, 424.

6. Levine, Max., 1916—"The Significance of the Voges-Proskauer Reaction," Jour. Bacteriology I, 153-164.

6. Clark and Lubs, 1915—"Differentiation of Bacteria of the Colon-Aerogenes Family by Indicators." Jour. Infectious Diseases, 17, 160-173.

6. Levine—"Correlation of the Voge roskauer and Methyl-red Reactions on the Colon-Aerogenes group." Jour. Infec. eases, 18, 358-367.

6. Levine—Private Communication.

7. Clarke and Lubs, 1915—"Differentiation of Bacteria of the Colon-Aerogenes Group." Jour. Infectious Diseases, 17, 160-173.

8. Bohme, 1905—Centrall. fur Bakt, Abt. I, Orig. XL, 129-133.

9. MacConkey, 1909—Journal of Hygiene, 9, p. 91.

9. Hollman, 1914—"Decolorized Acid Fuchsin as an Acid Indicator in Carbohydrate Fermentations." Journ. Infec. Dis., 15, 227-233.

10. Escherich, 1886—"Darmbak. des Sauglings, Stuttgart."

11. Migula 1886—"System der Bakt," 396.

12. Escherich and Pfaundler, 1903—"Handbuch d. Path. Mikroorg," Kolle and Wassermann. Zweiter Band, 341-342.

13. Escherich and Pfaundler, 1903—"Handbuch d. Path. Mikroorg," Kolle and Wassermann. Zweiter Band, 406.

15. Houston, 1905—"Bacteriological Examination of Milk." Special Report, London County Council, 37.

16. Durham, Journ. Exp. Med., V., 354-388.

17. MacConkey, 1909—"Lactose Fermenting Bacilli." Journ. Hygiene, 9, 88.

18. Ellis, 1904—"Discovery of Cilia in the genus Bacterium." Centrl. fur Bakt. II, 241-251.

19. 1904—"Report of the English Commission." Jour. State Med. XII, 471.

20. 1913—Prescott and Winslow. "Elements of Water Bacteriology," Wiley, 104.

21. Savage, 1906—"Bacteriological Examination of Water." Lewis, 82-83.

22. Migula, 1900—"System der Bakterien," 734.

23. Emmerich, 1885—"Untersuch. ub. die Pilze d. Cholera asiastica." Arch. fur Hyg., Bd. 3.

Macé (full description) 1891—"Traite Pratique de Bacteriologie." Paris, 498.

24. Jackson, 1911—"Classification of the B. coli Group." Journ. Infec. Dis., 8, 241.

25. Jordan, 1916—"General Bacteriology." Saunders Co., 275.

25a. Loq. cit., 273.

26. Levine, 1916—"Preliminary Note, Classification of the Lactose Fermenting Bacteria." Journ. Bacteriology, I., 619-621.

27. MacConkey, 1905.—"Lactose fermenting Bacteria in Faeces." Journ. Hyg. 5, 333-379.

28. Harrison, 1917—Private communication.

29. Jordan, 1890—"Report Mass. State Board of Health." 836.

30. Rogers Clarke & Evans, 1915—"Charact. of Bacteria of Colon type occurring on Grains." Journ. Infec. Dis., 117, 137-159.

31. Besson, 1913—"Text Book Practical Bacteriology, etc." Longmans, 442.

32. Jordan, 1917—"Differentiation of the Paratyphoid-Enteritides Group." Journ. Infect. Diseases, 20, 456.

33. Savage, 1913—"Bacterial Food Poisoning and Food Infection." Med. Off. Report, Local Govt. Board, 1913. Food Reports, No. 18, 33.

34. Winslow, C.-E. A. and A. R., 1908—"The Systematic Relationship of the Coccaceæ." Wiley, New York, 161, 169, 170.

35. Andrews & Horder, 1906—"A Study of the Streptococci pathogenic for Man." Lancet, II., 708.

36. MacCallum, W. G. & Hastings, T. W., 1899—"A case of Acute Endocarditis caused by M. Zymogenes." Journ. Exp. Med. IV., 521.

37. Birge, 1905.

37. Birge, 1905—"Some Observations on the Occurrence of M. Zymogenes." Johns Hopkins Hosp. Bull. XVI., 309.

38. Escherich Migula, 1900—"System der Bakterien," 31.