BACTERIAL DESTRUCTION

OF COPEPODS, & ONE OTHER

STUDIES IN MARINE BACTERIOLOGY.

1. BACTERIAL DESTRUCTION OF COPEPODS.

2. THE BACTERIOLOGY OF SWELLED CANNED FISH.

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I.

BACTERIAL DESTRUCTION OF COPEPODS.

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BACTERIAL DESTRUCTION OF COPEPODS.

During the summer of 1916 I was investigating the bacteriological content of "Swelled Canned Fish" forthe 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, the report of the detailed studies of these organisms is presented.

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% sodium indigo sulphate with 2% glucose added to neutral agar, tubed and sterilised in flowing steam for three successive days.

Tochte rmann's Serum agar. (2) For digestion test.

Koeffler's Blood Serum. (3) " " "

Aesculin agar. (4) For specific reaction of organism of the colon aerogenes group. Loops of a broth culture spread on plates.

Neutral Bile Salt Agar. (5) ditto. ditto.

Bouillon for Voges Proskauer reaction. (6)

Bouillon forthe 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% peptone, 5% NaCl dissolved in distilled water, the reaction adjusted to + 10, medium cleared with white of egg, filtered, tubed and sterilised. 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% solutions in distilled water, and ster-

-ilised for 15 minutes in flowing steam for three successive days. Immediately before inoculation these were added to tubes of broth made up as per 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% sugar or other carbonydrate 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%.

In the preparation of the indicator I have noticed as reported by Amdrade and Hollmann 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 decolorise to the proper shade of amber 100 cc. fuchsin solution.

Litmus Milk. The milk freshly separated and tubed was sterilised for three successive days for 30 minutes in flowing steam. The litmus was made up separately; a 7% solution of "Merck's" litmus in distilled water, heated in the steamer for 30 minutes and left over night in the incubator, filtered, sterilised 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 17 that Culture 3 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 mm. long and 1 mm. 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 3. No motility.

CULTURAL CHARACTERISTICS.-

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

Glucose Agar 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.

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

Sodium Indigo Sulphate Agar Slope. Luxuriant, raised, moist, spreading, noreduction. 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 hrs. Clouding abundant, medium clearing, flaky sediment at bottom, bluish rim at top. In three days flocculent yellowish-white rim at top, easily dislodged on shaking. Medium almost clear.

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

Milk. Coagulation in 24-30 hrs.; 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 coagulation 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 hrs. 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 mn. exposure in nutrient broth at 60°C.

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

Vitality on Culture Media. Active cultures have been recovered from agar after 5 min. at temperature of 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) 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 (1 and 2) 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 "flaginac" 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 give 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 Standardisation 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) 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 6) is the fermentation of saccharose to acid and gas. B. coli (Escherich) (10) has no action upon saccharose. Theobald Smith, cited by Bescott 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 organism 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); positive to lactose, saccharose and dulcite as B. communior (Durham); positive to lactose

and saccharose but negative to dulcite as B. aerogenes (Escherich); positive to lactose but negative to saccharose and dulcite as B. acidi-lactici. Further subdivision according to the action on mannite and raffinose are used for further differentiation.

MacConkey (27) 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. aerogenes 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 Král, and out of 480 coli-like organisms isolated from human and animal faeces he found that 23% gave biochemical reactions identical with the Král culture used by himas control. He states that he cannot agree with Migula in describing B. neapolitanus (Emmerich) as identiaal 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 is 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. neapolitanus 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 and Escherich 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 of a lactose fermenting organism. Later work already referred to has separated these two strains on the basis of saccharose fermentation (~~24~~). 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 characterise the organism I have isolated, a non-motile, lactose, sabharose, dulcite positive, Voges Proskauer negative strain, as a variety of the sub-type B.neapolitanus of the classic B.coli type of Escherich. (2~~6~~) ~~(27)~~. 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 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.

MORPHOLOGY. Microscopically - 24 hrs old agar culture at 37°C. - rods varying up to 1.6 mm. long and .8 mm. 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 hrs 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.

Loeffler's Blood Serum. Moderate, filiform, moist, glistening, no liquefaction, no discoloration. In 7 days no digestion, no discoloration.

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 reactiona, 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 contured, centre dark with paling towards edges. Deep surface colonies gramular centre with dark concentric rings.

Agar colonies. 24 hours at 37°C. - surface 1 mm. diam. 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 anarobe; 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 2 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 experiance 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,footnote). 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 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-aerogenes group. Taking the classification adopted by the American Public Health Association (1) the culture would be ruled out of the colon-aerogenes 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 whose 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 agglutinated by immunising 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-aerogenes group as lately given by Levine (27), 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. mm., 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 agitationcan be noticed, and rotation of the cells as on an axis, but the position in the drop is unchanged.

CULTURAL CHARACTERISTICS.-

Agar Slope. 24 hrs. 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.

Loeffler'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 hrs. 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 hrs. In 3 days faint growth, flat, spreading, white, metallic lustre.

Milk. 37°C. In 36 hours weak coagulum, no gas noted. In 72 hrs 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 disintegrated 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 3 . If litmus be added at the rate of ½% coagulation preceded by bleaching takes place within 36 - 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 hrs. 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 ½% the reaction is quite diff erent, 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 hrs 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 anarobic 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.	Mamite.	Dulcite
+	+	+	+	+	- -

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 characterised by Andrews and Horder (35).

I find, however, a closer resemblance to an organism described by MacCallum and Hastings (29) as Micrococcus zymogenes. This was isolated from a fatal case of acute endocarditis, and

while it shows the same main characteristics as Str.gracilis, it 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 copepods which had died in culture flasks.

2. Summarised 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 diges.	Slight diges.
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. Summarised, 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.

I wish to thank Dr. A.B.Macallum for permission so readily granted to present a note elsewhere on certain features of Culture III; Dr. F.C.Harrison for his kindness in reading the proofs, and particularly for his valuable and critical assistance with regard to the classification of the B.coli group; and Dr. Arthur Willey for the initial suggestion that I should undertake the investigation.

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II

THE BACTERIOLOGY OF SWELLED CANNED FISH.

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THE BACTERIOLOGY OF SWELLED CANNED FISH.

In a survey of the literature relating to the bacteriology of "Canned Fish" it is found, with a few exceptions, that the investigations recorded have been undertaken in connection with proved and alleged cases of food poisoning. Consequently the data available is largely interrelated with data on the bacteriology of canned meats, and of ordinary meats as supplied unpreserved. The exceptions of which I have knowledge are the investigations of Prescott and Underwood (1897) on "Microorganisms in the Canning Industries"; the work of Macphail associated with Bruére (1897)[2] on "Discoloration in Canned Lobsters"; and the recent work of Obst on "A Bacteriological Study of Sardines" 1916.[3] Prescott and Underwood working on cans of spoiled clams and lobsters, isolated species of bacteria, two classed as micrococci, the other seven as bacilli. The investigators found the cans to be badly decomposed, in some cases almost entirely liquefied, much darkened in colour and of a very disagreeable odour.

Of the bacilli, six coagulated and digested milk, while none of the seven produced gas in sugar solutions. According to the description given certain of these cultures bear a close resemblance to some recorded by me among the organisms in Class II on page 59. Both strains of micrococci isolated by these workers failed to coagulate milk and failed to produce gas in sugar solution. The bacteria were not named.

Macphail and Bruère[2] in their work on lobsters, isolated and recorded the features of four strains of bacteria; two were cocci, and two were fine rods. Each of the four were inoculated into sterile cans of lobster, and in due course the rules of proof were satisfied. Some of the organisms I have isolated, bear a resemblance to certain of the strains described by Macphail and Bruère; but it impossible to express a definite opinion as to their mutual identity.

Obst[3] in the report of her investigations on "A Bacteriological Study of Sardines" states that a bacillus, designated "Bacillus A", has been found in pure culture in two hundred and eighty seven swelled sardine cans. The organism is, a gas-producer & a spore-former* and according to Obst is possibly identical with B. Walfischrauschbrand (Ivar Nielsen)[4].

The only reference I can find to the bacillus of Nielsen[4] fails to give full cultural details. In the fall of last year I was in communication with Miss Obst, but at that time her report was not available; as I have received no copy I consider it probable that it is not yet published. From the reference cited[3] which abstracts a recent paper read before the Society of American Bacteriologists, I am unable to compare any of my strains with the "Bacillus A". The reference does not mention the thermal death point in laboratory media, but states that the organism, under certain conditions survives bathing in boiling water for 1½ hours. With the strains described in my report no experiments in commercial conditions have yet been conducted. For the present I am not justified in stating further than

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* In the strains I have isolated, no evidence of spores has been demonstrated.

~~to state~~ that, based on such information as is available it is improbable that the strains isolated by me, are identical with the "Bacillus A" of Obst.

The relationship of bacteria to sardines was discussed by Auche[5] (1894) but the paper is not available.

The association of mussels with food poisoning is cited by Vaughan, 1892[6]; citing from Vaughan's paper :-

> "That chemical poisons may be transmitted from the lower animals to man in the food is shown by the history of poisoning with mussels and with fish. As early as 1827 Combe described in detail the symptoms induced by the eating of poisonous mussels, and a valuable contribution to the same subject has recently been made by Schmitdmann, who has found that non-poisonous mussels placed in the water of Wilhelmshaven soon became poisonous, and that the poisonous mussels from the harbour soon lose their harmful properties when placed in the open sea. Linder has found in the water of this bay and in the mussels living in it a great variety of protozoa, amoeba, bacteria, and other low forms of life, which are not found in the water of the open sea, nor in the non-poisonous mussel. He has also found that if the water of the bay be filtered, non-poisonous mussels placed in it do not become poisonous. He therefore concludes that poisonous mussels are those which are suffering from disease due to residence in filthy water."

In view of the close relationship to mussels of clams, a variety of shell-fish canned in both New Brunswick, and Maine, U.S.A., the observations of Linder cited by Vaughan are of considerable interest. In the same paper, Vaughan describes the case of one of his own patients who showed poisoning symptoms after eating freely of canned salmon. The patient under treatment recovered. Vaughan submitted the remains of the salmon to various tests and found an organism which he describes as follows :-

> "The only germ which could be found, either by direct microscopic examination or by the preparation of plate cultures, was a micrococcus, and this was present in the salmon in great numbers. This germ grew fairly well in beef-tea, but the injec-

-tion of five cubic centimeters of the beef-tea culture of different ages failed to affect white rats, kittens or rabbits. However, this micrococcus when grown for 20 days in a sterilized egg, after Hueppe's method of anaerobic culture, produces a most potent proteid poison. The white of the egg becomes thin, watery, markedly alkaline, and 10 drops of this suffices to kill white rats.
Evidently in the preparation of the salmon this can was not sterilised; it was sealed, and for months, possibly longer, this germ had been growing anaerobically, and elaborating a chemical poison."

Savage in England who has investigated many outbreaks of food poisoning has isolated B. enteritides (Gaertner) from tinned salmon. Griffiths cited by Vaughan (Gaertner) and Novy[8] claims to have isolated a ptomaine, sardinin from sardines.

In view of the types of bacteria I have isolated in the present investigation, it is of importance to note that Poels[8] in Rotterdam has isolated varieties of B. coli from cases of food poisoning due to the eating of meat from a supposedly healthy animal. McWeeney[9] considers that meat poisoning outbreaks are due to organisms of the following groups :-

(a) The Typho-coli group, including B. enteritides (Gaertner)

(b) The group of putrefactive aerobes (Proteus etc.)

(c) The obligate anaerobes (B. botulinus).

It will be seen, page 54, that of the organisms I have isolated, some strains are varieties of the Proteus group, and some varieties of the B. coli group. Vaughan and Novry[8] described the most common form of food poisoning, that caused by contamination of foods with saprophytic bacteria; such bacteria either before or after the food has been eaten, elaborating chemical poisons.

PRESENT INVESTIGATION.

The investigation herein described of the "Bacteriology of Swelled Canned Fish" has been undertaken on behalf of the Biological Board of Canada. The work was commenced in the summer of 1916 at the Marine Biological Station, St. Andrews, N.B., and has since been continued in the laboratories at the College. To the canners, the appearance of "swells", as they are termed, in the cases of canned fish sent out from the factories, is a matter of considerable concern. The desirability of undertaking experimental work in the hope of eliminating any risk of cans developing the swelled condition, suggested itself to the Principal of this College, Dr. F.C.Harrison, in the summer of 1915. At that time Dr. Harrison was engaged at the Marine Station, St. Andrews, in the examination of salmon attacked by a bacterial disease; and it was while conducting this investigation, that the problem now under discussion came under his notice.

The matter was brought to the attention of Dr. A.B.Macallum Secretary of the Board, and in due course it was my good fortune, to be asked to take up the work. The procedure to be adopted was left entirely in my own hands. Dr. Macallum, and Dr. A.G.Huntsman, Curator of the Marine Station at St. Andrews, have throughout given me every encouragement, and the greatest possible help in every way which seemed likely to assist in the elucidation of the problem.

On arriving at the Station in July, the necessary arrangements were made by Dr. Huntsman enabling me to visit a number of the New Brunswick canning factories. Later it was made possible

for us to visit several of the largest plants operating in the State of Maine. I was thus brought into close touch with the industry of canning as a commercial undertaking, had exceptional opportunities of seeing the methods of packing as generally adopted, and accumulated a store of information as a result of discussions with the canners themselves. Factories were visited which were engaged in the canning of herring, sardines, haddock, and clams respectively. It is hardly necessary to say that the sardines of New Brunswick and the State of Maine are small herrings. It was apparent that the canning factories were principally concerned in the packing of sardines; and while both during the summer, and since returning to the College, swelled cans of sardines, herring, haddock, lobster, and shrimps have been gradually accumulating, the work has up to the present been confined entirely to sardines and possible influences affecting the same. After nine months work I find that I have been able to do little more than touch the fringe of the problem considered as a whole. The report here presented therefore is principally concerned in recording the work accomplished up to the present, such conclusions as it is legitimate to draw at this early stage, and such information as to methods and media used in the laboratory as will make the work of some service to the continuance of the investigation.

Under the circumstances I do not propose to enter into a detailed description of the equipment, methods of treatment, and system of packing of the fish, and general procedure of the factories engaged in the canned fish industry; suchwill be more appropriate when the work has progressed to a more advanced stage.

The one phase of the canning process of which brief mention must be made at this point is the temperature employed in the so-called sterilisation of the cans when packed and finished. As the most common size of can produced from all the factories is one weighing from 3 to 4 oz., the temperatures given shall be those applied to cans of this size.

In the majority of the factories visited, the cans are immersed in baths of boiling water for a period of $1\frac{3}{4}$ hours. That completes the heating process. Briefly the essentials of the treatment of the fish-which have been salted in the boats as taken from the weirs, on arrival at the factory, is as follows :- immersed in a mixture of sea water and salt for 1 - $1\frac{1}{2}$ hours; spread on racks, termed flakes, in thin layers, and for 10 minutes placed in flowing steam; dried in room through which hot air is continually circulated for 1 hour; heads discarded and the remainder of the fish arranged in the cans; oil automatically added, and tops put on and fastened by either the "rolling" or the "pressing" process. The cans are then heated as specified above.

In some factories the preliminary steaming for 10 minutes is dispensed with, and a continuous progression through a bath of cottonseed oil at a temperature of 200°C. is substituted, this occupying 2 - 3 minutes. In such factories the final heating is as above, $1\frac{3}{4}$ hours in baths of boiling water.

In one factory where the fish are fried in oil for 3 minutes or so, the final heating is done under pressure at a temperature of 225°F. for a shorter period.

It should be added that in all the sardine factories visited, the most careful supervision is exercised in the final packing of the cans in cases before shipping. Each individual can is rapidly passed through the hands of an expert "tapper" who discards cans displaying any irregularity, such being reprocessed or entirely discarded.

The project of the investigation may be logically stated thus :- "Essentially to determine whether or not the swelling of the cans is due to the activities of bacteria."

If on examination, and when submitted to suitable cultural methods, strains of bacteria are isolated, the procedure to be as follows :-

1. Purify and obtain in pure culture.
2. Determine the morphological, biological and biochemical characteristics of the organisms.
3. Inoculate the strains obtained in pure culture into normal cans, and record condition at stated intervals.
4. Treat "control" normal cans in a similar manner except for the inoculation with the culture.
5. If swelling occurs in the inoculated cans and no change is noted in the "control" cans the presumption is raised that the swelling is due to the organisms used for inoculation.
6. Examine the experimentally "swelled" cans and determine in culture the presence or absence of bacteria.
7. If bacteria are found, purify and compare culturally with the strains used for inoculation.
8. If on comparison the strains be found culturally identical, the cause of the "swelling" has been established; and experimental proof has been obtained to warrant the statement "that the swelling of the cans is due to the actitities of bacteria."

The data recorded in this report show that up to this point the work has been successfully accomplished in so far as concerns certain strains of bacteria; and the "Postulates of Koch" have been satisfied.

While at the Biological Station, I not only visited the factories as already stated, but many swelled cans of sardines were secured and a number of organisms in the cans isolated in culture. An attempt was also made to discover the source of the organisms. Samples of sea water taken from the weirs, samples of oil and tomato sauce as used in the packing, intestines of fresh herrings and the excreta of herrings were obtained.

No organisms were found in the oil; the tomato sauce in sealed receptacles as imported from Italy has still to be examined; but from the sea water, herring intestines, and herring excreta several strains of bacteria were isolated. These, with those I found in the sardine cans, I brought back on my return to the Laboratory here. During the succeeding months a number of the cultures have died out, and those remaining from sea water, herring intestines, or excreta all fail to produce gas in carbohydrates.

For the sake of convenience I have divided the strains of bacteria isolated at St. Andrews and at various times during the fall and winter into two main classes :

Class I Gas-producers.

Class II Non-gas-producers.

For obvious reasons, my attention has been principally confined to the gas producers, Class I., and it is to the descriptions of these that the cultural part of the report is chiefly directed.

Regarding the influence of these organisms included in Class II on the condition of the fish in the swelled cans, I am not in a position to express any opinion. I have, however, submitted many of them to certain preliminary tests, the results of which are recorded,- Pages 54-65. Beyond this I have not gone, and no comments respecting the class are made.

Concerning the gas-producers, Class I, 8 strains have been described morphologically, biologically and biochemically. The detailed descriptions are found on pages 21-52

On pages 52-53 a summary arranged in tabular form is shown.

The number of cultures described in Class I, and those more briefly referred to in Class II, bear no relationship to the total number of cultures isolated in the course of the work. As was to be expected, preliminary tests of a differential nature revealed the fact that many strains were in duplicate and sometimes even in triplicate. By repeated series of tests the duplicates or triplicates were gradually eliminated. In the pages devoted to the cultures in Class II, pages a note is added as to the comparative frequency of the respectivestrains. In eliminating strains from the cultures in Class I, greater precautions were taken on account of their closer relationship to the abnormal condition of the cans; some of the final cultures described representing the individual strains, after the elimination of as many as four or five strains had been found having the main characteristics in common; further, three cultures of Class I were finally eliminated to avoid duplication in description, just prior to the preparation of the manuscrips, these being identical with Cultures 34, 37 and 64 respectively.

To continue the statement as to the project of the investigation, initiated on page , it is further required that in order

to confirm the work up to the present and complete the investigation it is desirable :-

9. That many more cans shall be examined and the contents cultured.

10. That if possible the source of the responsible organisms be determined, and also the stage at which infection takes place.

11. That experiments be conducted both under laboratory conditions, and under conditions prevailing in the canning factories, with a view to determining the most satisfactory means of eliminating "swelling."

12. That possibly the pathogenicity or degree of pathogenicity of the strains proved responsible for the "swelling" be determined by inoculation into suitable laboratory animals.

Arrangements have been made by Dr. Huntsman whereby during the coming summer I shall have opportunities of determining if possible the source or sources of the causal organisms of the swelled condition of cans of sardines.

The future scope of the laboratory work will necessarily include examination of swelled cans of other varieties of fish, including those of which mention is made on page 9 .

When visiting the canning factories last summer the manager of one of the largest of these told us that a pressing problem with which he had to contend was the frequent appearance among sardine cans of what are termed "sour flats." The condi-

tion is one of which there appears at present to be no satisfactory explanation. The product is rendered unmarketable, and the condition is one which cannot be detected until the cans are opened.

MEDIA EMPLOYED.

In this investigation I have used media prepared from fish concoctions, the ordinary laboratory media, and certain special media. In the early part of the work when experimenting with methods prior to the adoption or a definite procedure, difficulty was experienced in growing some of the strains isolated. The colonies developing on some of the plates at this time were too small to be subcultured. I therefore utilised the marine resources at hand and prepared media from fresh herrings, from clams, and from seaweed, using fresh sea water instead of tap or distilled water. It was found later that the organisms which necessitated this media were those I have put in the Main Class II, the non-gas-producers. After successive subculturing in the laboratory these same strains have grown moderately well on the usual standard media.

The organisms of the main Class I, the gas-producing strains, have grown well in the standard media. The growth of some strains has been more luxuriant on herring media or clam media, but I have gradually eliminated the use of such for two reasons :

(1) the satisfactory growth obtained on standard media, and the convenience of its use;

(2) the necessity of using the standard media in order to compare the strains isolated with varieties already described in literature.

HERRING BROTH : Fresh herrings obtained direct from the weirs were washed in running water and ground up, no portions discarded, through a meat grinder, mixed with sea water, 1 part ground herring to 1-2 parts sea water,

and heated for several hours in the steamer or autoclav. The mixture was allowed to cool and the fat skimmed off; again heated and strained through cheese cloth. The strained liquid served as the standard herring extract. Varying strengths of broth were made up, good results being obtained from the following mixture :-

500 cc. standard broth.
1000 cc. sea water.
15 grams peptone.

The ingredients were heated together in the steamer, neutralised with n/20 NaOH to + 10 (phenol phthalein indicator) cleared with white of egg, tubed and sterilised in the usual way.

HERRING AGAR : To 500 cc. of the standard broth, mentioned above, were added 500 cc or 1000 cc. sea water, peptone at the rate of 1% and agar at the rate of 1.2%; the whole heated together until ingredients dissolved, neutralised to + 10, cleared with white of egg, filtered, tubed and sterilised in the usual way.

CLAM AGAR : Fresh clams were dug up on the beach, washed in running water, opened and ground through meat grinder; to this was added sea water at the rate of 1 part clams to 2 parts sea water, and the whole heated for several hours in steamer or autoclav. The stewed mixture was strained through cheese cloth; this filtrate constituting the standard broth. To 500 cc. of the standard broth

were added 1000 cc. sea water, peptone at the rate of 1%, and agar at the rate of 1.2%; the whole heated together until ingredients dissolved, neutralised to + 10, cleared with white of egg, filtered, tubed and sterilised in the usual way.

I have also steamed clams in the shell in sea water, approximately weight for weight; retaining the juice which has a typical 'sheen'; then after opening the clams using them as described above.

In the earlier part of the work the medium was used successfully to some considerable extent; and in comparison with standard beef peptone agar it appeared to exercise a selective action towards certain strains of bacteria obtained from various sources. This in all probability would be due to the glycogen content. While the use of this medium has for some time been discontinued, I propose to test its value for certain phases of the laboratory analyses.

Baur[10] in working at Kiel on the denitrifying bacteria used and recommends a broth of which mussels are the essential component.

BEEF PEPTONE AGAR : Standard methods.[11]

BEEF PEPTONE GELATINE : Standard methods.[11]

GLUCOSE AGAR : 1% glucose added to agar prepared as above, immediately before tubing.

LOEFFLER'S BLOOD SERUM :[12]

LOEFFLER'S TYPHOID SOLUTION :[13] This medium containing malachite green has been recommended by Loeffler for use in culturing

strains of the colon-paratyphoid-typhoid group.

AESCULIN AGAR (14) : For specific reaction of organisms of the colon-aerogenes group; loops of a broth culture spread on plates.

MACCONKEY'S NEUTRAL RED BILE SALT LACTOSE BROTH: (15) For reduction test of organisms of the colon-aerogenes group.

BOUILLON FOR VOGES PROSKAUER REACTION : (16)

BOUILLON FOR METHYL RED REACTION : (17)

SOLUTION FOR REDUCTION OF NITRATES TO NITRITES : (18) Giltay's synthetic solution was used.

DUNHAM SOLUTION FOR INDOL PRODUCTION. (19)

GLUCOSE BROTH : 1% glucose in Dunham solution. (19)

FERMENTATION BROTHS : For the fermentation reactions I have used ten test substances. It will be seen that in addition to the glucose salicin I have adopted the use of another glucoside Aesculin - used in conjunction with iron citrate by Harrison and Vanderleck - as a fermentable test substance in Dunham broth. I have been using aesculin for this purpose during the last four months in connection with work on the gas producing organisms in the Ottawa river water, and find a correlation in the black reaction of the aesculin agar medium, and the production of acid and gas in aesculin used as a carbohydrate test substance.

LITMUS MILK : (20)

METHODS.

On account of the comparative paucity in the literature, of descriptions of actual methods adopted in the isolation of bacteria from swelled canned fish, the procedure I have followed has largely been determined by experience as the work has progressed. This procedure has been changed as better methods suggested themselves; and in the culturing from the many cans still awaiting examination, I propose further changes affecting detail; while the use of additional media which will be to the advantage of the work has suggested itself.

ISOLATION OF BACTERIA FROM THE CANS.

The oily greasy surface characteristic of the cans with pronounced swelling, necessitated the use of a disinfecting agent which would disinfect and remove the oil at the same time. Absolute alcohol has proved to be simple in application, and quite satisfactory. The cans were first cleaned with a weaker alcohol (70% to 90%) then thoroughly treated with the absolute alcohol. Can openers, forceps, and dissecting scissors were immersed in alcohol and flamed immediately before use. When a sufficiently large aperture had been made in the can, pieces of fish and a portion of the oil or sauce were removed with forceps and pipettes, and inoculated into tubes of liquid medium.

At the commencement it was at once obvious that direct plating from the cans would not be at all satisfactory on account of the oily nature of the contents; liquid media have therefore been used for the ~~prime~~ first inoculation from the cans, the procedure having the additional advantage in that such media serve as enrich-

ment fluids. I ~~first~~ first used, peptone broth (Dunham), herring broth and nutrient broth; later, the addition to the series of glucose peptone broth proved to have advantages. As a result of the additional knowledge provided by the ~~starins~~ strains of organisms already worked out, it will be desirable in the further work to use media having differential qualities for the ~~prime~~ first inoculations; in addition to the broths already in use.

The tubes were incubated at 37°C. except during the six weeks spent at St. Andrews, when all ~~incubations~~ cultures were kept at room temperature. The broths were examined in 18 - 24 hours for clouding; if no growth was apparent further incubation was resorted to; if growth ~~could be~~ noted, series of plates were made. The preliminary incubation in both tubes had the additional advantage to those already mentioned, in that the oil had risen to the surface leaving the subsurface liquid comparatively free. Finely drawn out pipettes ~~were lowered,~~ were with the finger over the end, passed through the layer of oil and the culture fluid drawn up. After suitable dilutions had been made, plates were poured using herring agar, clam agar, beef peptone agar, and glucose agar; in the more recent work glucose agar being used almost solely. The plates were incubated - temperatures as aforementioned - and when growth was sufficient those colonies most common were streaked on agar slopes, from these the necessary purification by plates being made.

NOTE: The preliminary incubation in broth tubes was in some cases, but not always, duplicated aerobically and anaerobically.

* Reaction to Gram's stain The gas-producing organisms
species in this report as Class I, each
showed great resistance to the decolorization
by alcohol. Following the usual procedure of
washing out with alcohol until no more color
washes from the slide, the organisms would have to
be classed as Gram positive; the violet color being
a little less deep than the typical gram positive
reaction, but more nearly positive than negative.
When the slides were soaked in absolute alcohol for
30-40 minutes decolorization was as complete as the
common gram negative reaction. As control, films were
made from an agar culture of a typical gram-positive
lactic acid producing organism. These slides without
soaking in absolute alcohol for 50 minutes were then
typically gram-positive films.

The organisms are therefore, classed as
Gram-negative showing an unusual resistance to the
decolorizating by alcohol

THE FOLLOWING APPLY TO THE MAIN CLASS I :

MICROSCOPIC EXAMINATIONS : The microscopic preparations were uniformly made from beef peptone agar slopes incubated 18-24 hours at 37°C.

MOTILITY : Hanging drops for these tests were made from the water of condensation, agar slopes; young cultures incubated at 37°C. never longer than 24 hours.

INOCULATION OF MEDIA : All tubes of media used for the determination of cultural features and biochemical reactions were inoculated from young peptone broth cultures of the particular organism. The use of peptone salt solution instead of nutrient broth eliminated to a minimum any risk due to the presence of muscle sugar. It may be mentioned that repeated tests for the presence of muscle sugar in the peptone used, gave a negative reaction based on the absence of acid and gas; the tubes being inoculated from an active strain of the B.coli group.

Prior to the inoculations of the series, peptone broth tubes were inoculated from agar slopes, and incubated at 37oC. After 18-24 hours, usually about 20 hours, the whole series would be inoculated with the broth from a 1 cc. pipette; 2-3 drops of

culture to each tube. Slopes of solid media were streaked with a standard 3 mm. loop platinum needle. The number of tubes involved, and the amount of test substances necessitated, have been considerable throughout the work, and to insure economy of expense and time, strictly quantitative estimations of the gas evolved have not been carried out other than by means of the Dunham tube. In view of the method noted above, however, the results are truly comparative throughout. Moreover, for the particular purpose of the present work the essential point to be decided regarding the fermentation of the test substances to gas is this - does a particular culture produce gas, or does it not produce gas ? It is not only of considerable interest, but of much practical and classificatory value to know whether the amount of gas produced in a given time at a given temperature in a given substance is great or small; ~~Again,~~ such information can be comparatively well shown by the use of the Dunham tube.

INDOL PRODUCTION: The tubes to be tested for Indol were incubated at 37°C. for 7 days; the Bohme Ehrlich test being used.

REDUCTION OF NITRATES: The Giltay solution was tested after 3-4 days incubation at 37°C. for the presence of nitrites. The sulphanilic acid and a-napthylamin reagents were used.

VOGES PROSKAUER REACTION: After 48-72 hours incubation at 37°C. the cultures tested with a strong solution of K.O.H. The test if positive has usually shown the typical eosin shade in the upper layers within 2 hours at room temperature.

METHYL RED REACTION: Determined after incubation at 37°C. for 48 - 72 hours.

CANS OF SARDINES.

General Description.

Appearance of Cans and Conditions of Contents.

Owing to the varieties of 'Brands' of sardines produced by the canning factories, the various methods of packing adopted, and the different substances utilised for the giving of flavour ~~and consistency~~ to the finished product, it is not possible other than in a general way, to describe the conditions met with in my examinations.

NORMAL CANS.

In outward appearance there is a complete absence of any 'bulging'; the top and bottom are either quite flat or almost imperceptibly concave. On shaking there is no 'rattle' and scarcely any movement of the contents can be heard. When opened with the cutter there is no expulsion of air or gas, with little if any exuding of the oil or other material used in the process of packing.

The contents are firm, not macerated, and often white in colour; this last, however, depending to some extent upon the materials used in the packing. The smell is mildly characteristic of the fish, qualified by the variety of oil or tomato sauce used. There is in appearance and odour a complete absence of putrefaction. The fish are saturated to a greater or lesser extent with the oil, sauce, or other flavouring agents used, but without losing their firm and solid condition. The oil or sauce will be seen asa layer over and in the ~~interstices~~ interstices between the individual fish rather than actually within the bodies.

SWELLED CANS.

Outwardly the cans vary from a slight 'bulged' appearance to a more pronounced swelling. The top and bottom are forced out as a result of the pressure and present a decided convex surface. As the swelling becomes greater the oil or sauce will be forced out between the soldered parts of the can; and in pronounced cases the outside surface is greasy and wet, and possibly covered with the oil or sauce. Swelled cans when shaken have a characteristic 'rattle' on account of the extra space within resulting from the swelling. When the cans are opened gas is expelled, accompanied in advanced swellings by portions of the liquid contents. In advanced cases there is a tendency for the oil or sauce to pour out over the surface of the cans.

The condition of the contents varies considerably. Usually the fish are macerated, disintegrated and soft, and are intermixed with the oil or sauce; they have lost their entity. The odour is variable,- frequently it is not unpleasant, resembling to an accentuated degree the natural smell of normal sardines. In other instances a pronounced putrefactive odour is evident. It may be that the putrefactive odour is present at all times and is masked by the spices or other ingredients of the sauce. That is a point which can only be definitely pronounced upon after a more extended investigation.

CANS EXAMINED.

Up to the pr esent I have examined forty cans, normal and swelled. The cans have been obtained personally or by express :

(1) direct from various canning factories in the province of New Brunswick, and in the State of Maine, U.S.A.

(2) From the Health Department of a city in the Maritime Provinces.

(3) From retail grocery stores.

Many of the normal cans, representative of the various factories, proved to be sterile; from some have been isolated spore forming bacteria, inactive on fermentable carbohydrates,- see page and in no instance have gas producing organisms been found.

From certain of the swelled cans I have isolated a variety of strains of gas-producing bacteria, none of which show evidence of spore formation. The cans from which these strains have been isolated are representative of three of the factories engaged in canning; and for the sake of ~~clarity~~ clearness these factories have been ~~specified as~~ designated Packer A, Packer B, and Packer C, respectively. Further, from swelled cans I have also isolated strains of bacteria which fail to ferment any of the carbohydrates used as test substances. It remains, ~~therefore,~~ to be added that from some cans obviously 'swelled' I have failed to isolate gas producing bacteria.

As already stated (page 9) the organisms isolated from the various sources have for the sake of convenience been arranged in two main classes :

Class I gas-producers,

Class II non-gas-producers.

The gas-producers (see page 24-58) have been isolated solely from swelled cans of sardines. Of the swelled cans examined the majority were obtained from sources 1 and 3 (page 44), some were submitted by source 2. Under the circumstances it has seemed desirable to use some means of differentiation. Accordingly the swelled cans obtained (1) from the canning factories, and (3) from retail grocery stores have been designated 'Swelled cans, Series I'; those submitted by (2) a certain City Health Department, 'Swelled Cans Series II.'

SWELLED CANS, SERIES I.

Can I. Packer B. Obtained direct from canning factory; packed with tomato sauce; characteristic 'swelled' appearance. The pressure of the gas was so great that on the can being opened part of the contents were strewn over the laboratory bench. The odour was pleasant though pungent, and may best be described as the natural smell of normal sardines accentuated. It is of interest to note that the ~~plates made using~~ herring agar plates, rapidly developed at room temperature a putrid smell resembling, as expressed by a laboratory colleague, that of an "oriental latrine."

See Culture 32, Class I.

Can II. Packer A. Obtained from a retail grocery store; packed in cottonseed oil; same brand as those of "Swelled Cans Ser. II". This can was passed as saleable and normal by a reputable salesman, and on personal examination of his stock I retained it as suspicious. I have no knowledge as to the date of packing. In appearance

the can was slightly swollen, convex, but there was no evidence of oil exuding due to pressure of gas. On opening, a perceptible amount of gas was forced out. The contents were soft and disintegrated; colour slightly darker white, than normal; odour an accentuation of the normal.

See Culture 34, Class I.

Can III. Packer A. Source and brand as Can II of this series. This can submitted to me by the salesman. The appearance of the can, the appearance, condition and colour of the contents identical with description applied to Can II.

See Culture 35, Class I.

Can IV, Packer B. Source and brand as Can I of this series. In this can the swelling had not progressed as far as in Can I, and on opening, the gas was not so profuse. The description there applied to the contents and to the nature of the subsequent plates is equally applicable in this instance.

See Culture 36, Class I.

Can V. Packer B. Source and brand as Can I of this series. The extent to which the can had swelled, and the further descriptions used above for Can IV apply here.

See Culture 37, Class I.

Can VI, Packer C. Obtained direct from canning factory; packed in tomato sauce; characteristic 'swelled' appearance top and bottom convex. On opening a small amount of gas escaped. The odour was not unpleasant, and may be described as the natural smell of normal sardines accentuated. The contents of the can were not nearly so much disintegrated as noted in some previously mentioned, were somewhat dry and a little less hard than the contents of normal cans.

See Culture 64, Class I.

SWELLED CANS, SERIES II. PACKER A.

A cargo of sardines exported by Packer A had been sunk in a harbour, remaining under water for six weeks. When the cargo was salvaged a ~~proportion~~ number of the cans were visibly swelled. The local Health Department submitted a number of these cans for examination, as a result of which the cargo was condemned. Such cans, of course, do not represent the 'swelled cans' of commerce. As, however, their condition, and the nature of their contents appeared somewhat similar to the swelled cans obtained from other sources, the characteristics of some of the organisms isolated have been included in this report.

To differentiate from the swelled cans obtained direct from the canning factories and from retaile rs I have designated the salvaged cans as "Swelled Cans Series II". The brand of sardines of which this cargo consisted is one of the least expensive brands on the market; cottonseed oil is used.

Can II. On shaking, perceptible 'rattle' characteristic of the swollen cans. On opening with the cutter, escape of gas

and pronounced putrefactive odour; contents soft and disintegrated; colour dirty white with tendency to redness in inner portions.

See Culture 24, Class I.

Culture 14, Class II.

Can III. Characteristic 'rattle'; escape of gas and pronounced putrefactive odour on opening of can; contents soft and disintegrated, and of a dirty white colour.

See Culture 26, Class I.

Culture 16, Class II.

CULTURE 24.

Source : Can II, Series II, Packer A.

MORPHOLOGY : Microscopically : coccus forms to short thick rods twice as long as broad; average length .8 - 1 mm. Gram negative.* From old agar cultures no evidence of spores.

MOTILITY : In hanging drop occurring singly, in twos and in chains; some individuals with rapid movement, some having slow undulating motion.

CULTURAL CHARACTERISTICS :

Agar slope : 36 hrs. 37°C. - growth luxuriant, raised, glistening, iridiscent, yellowish-white by transmitted light.

Loeffler's Blood Serum.- 24 hrs. 37°C. moderate, yellowish white, no liquefaction.

Loeffler's Malachite Green Sol.: Green precipitate or weak coagulum at bottom of tube; this very slowly changes and within 14 days partially digested; liquid portion assuming brownish tint.

Gelatine Stab. Room temperature : liquefaction begins in 24 hrs. crateriform; in three days liquefaction on surface and along track of needle, crateriform to infundibiliform; growth very slimy on this medium; in 7 days yellowish cloudy stratiform extending 1 cm. from surface, remainder infundibiliform with heavy yellow flocculent sediment to bottom of tube. In 18 days liquefaction not yet complete; upper portion heavy milky even cloudiness, merging into layers of semitransparent cloudiness, the lower portion a heavy ferric yellow mass of precipitate.

Nutrient Broth : 24 hrs. 37°C. - heavy clouding with bluish rim; in 3 days flocculent flakes of bluish tint on sides of tube; in 5 days very heavy dense even clouding, watered silk appearance; this condition persists.

Herring Broth : Condition similar to above; very heavy growth; in 9 days a loop of the liquid showing decided iridiscent bluish sheen.

Milk : In 24 hrs. unchanged, except that much froth on shaking; in 3 days coagulated, soft curd, some whey expressed; in 9 days yellow digested fluid 2/3 of tube, remainder white soft curd; in 14 days rppiness noted, and medium almost entirely digested with slight amount

* See page 19a

of flocculent curd at bottom of tube; in 5 weeks almost wholly turbid yellowish digested fluid with slight jelly-like yellowish iridiscent floccument curd on base of tube.

Litmus Milk : in 24 hrs. much froth on shaking, violaceus for 1 cm. from surface, remainder paler; in 3 days partly coagulated soft curd, violaceus; in 9 days digestion proceeding, fluid yellowish; in 14 days blue rim at surface, medium 5/6 digested, reddish brown tint; in 5 weeks slight flocculence, curd at base of tube, remainder partially cleared and tinted dark purpureus to heliotrope.

Aesculin agar : 1 loop from peptone broth culture streaked on plates. In 24 hours growth but no definite black reaction; later assumes brown to black tint, moderate growth.

Aesculin broth : In 24 hours black reaction.

MacConkey's N.R.B.Broth : No reduction to canary yellow in 24 hours.

Gelatine colonies : (1st appearance) in 72 hours liquefaction well advanced; individual colonies up to 3 mm. diameter, round, saucer-shaped, entire edges; liquefaction typical of the proteus group. centre of colony dark white spot .25 mm. diameter, remaind-er of colony varying from clear space to fine precipitated granules. Under the low power objective opaque centre, edges entire; medium tinted green, and distinct earthy smell.

Agar colonies : 20 hrs. at 37°C. growth moderate, surface colonies round, concave, glistening, raised, distinctly radiate; by transmitted light young colonies bluish, older colonies becoming whiter, more opaque and darker in centre. Subsurface colonies small but well defined, white. Under low power objective surface colonies distinctly yellowish with entire edges; on focussing through, dense and dark; structure cannot be defined; smaller colonies dark centre, then pale yellow, and near the edges almost transparent. Subsurface colonies well defined, edges entire, yellow to dense.

TEMPERATURE RELATIONS :

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

Optimum temperature : cultures incubated at room temperature and at 37°C. grow well. Most satisfactory growth at 37°C.

Vitality on Culture Media : The culture survives several months in artificial medium, agar or gelatine.

RELATION TO OXYGEN. The culture is a facultative anaerobe; incubated for 36 hours under anaerobic conditions moderate growth on glucose agar, as discrete colonies along track of needle 1 - 2 mm. diameter; by transmitted light concave, dark white centres, paling to blue at edges. Growth is not so luxuriant as under arabic conditions.

BIOCHEMICAL REACTIONS :

Indol production : Indol not produced.

Reduction of Nitrates : Nitrates reduced to nitrites

Voges Proskauer reaction : Positive.

Methyl red reaction : Alkaline.

Fermentation of Carbohydrates : This culture does not rapidly ferment many of the carbohydrates. In 24 hours lactose is but feebly fermented to acid; saccharose, mannite and xylose are fermented to acid and gas with prpfuse frothing; arabinose and inulin give slight gas; while gas appears in glycerine only after a period of 72 hours. The remaining substances used are fermented moderately well in 24 hours to acid and to gas.

Glucose.	Lactose.	Saccharose.	Mannite.	Dulcite.
+ +	+ -	+ +	+ +	+ -

Adonit.	Raffinose.	Arabinose.	Xylose.	Salicin.
- -	+ +	+ +	+ +	+ +

Aesculin.	Glycerine.	Inulin.
+ +	+ +	+ +

+ = acid.

+ + = acid and gas.

CULTURE 26.

SOURCE : Can III. Ser. II. Packer A.

MORPHOLOGY : Microscopically, rods 1½ to 1¾ times as long as broad; average length 1.6 μ with many longer forms even in young cultures. Gram negative*; from old agar cultures no evidence of spores.
Microscopic preparations made from cultures of this organism incubated at the same and at different temperatures have shown much variation in morphology; successive plate culturing however has failed to show impurity.

MOTILITY : In hanging drop occurring singly, and in twos, sometimes side by side; longer forms noted; non-motile.

CULTURAL CHARACTERISTICS :

Agar slope : 36 hrs. 37°C. moderate, along track or needle, glistening yellowish-white by transmitted light.

Loeffler's Blood Serum : Growth slight after 72 hours. No liquefaction.

Loeffler's Malachite Green Solution : 24 hrs. 37°C., coagulated as soft junket like curd attached to sides and bottom of tube, green, with pale green liquid expressed. After 14 days no change.

Gelatine Stab.- Room temperature - in 3 days scant growth, filiform, no liquefaction; in 18days no change apart from increased growth, no liquefaction.

Nutrient Broth : 24 hrs. 37°C. moderate clouding, no pellicle, no sediment, no ring; in 3 days watered silk appearance; in 9 days no change except slight sediment at bottom of tube.

Herring broth : Similar to above but much more luxuriant growth.

Milk : In 24 hrs. at 37°C. no coagulation, much froth on shaking; in 3 days coagulation beginning; in 5 days from coagulum, no gas, no digestion; in 16 days curd slightly split by gas. In 5 weeks shrinking of curd but no digestion.

Litmus Milk : In 24 hours violaceus, much froth on shaking, no coagulation; in 3 days lilaceous with weak coagulum; in 5 days curd slightly cracked by gas. In 5 weeks no digestion; pale lilac to isabella.

Aesculin agar : One loop from peptone broth culture streaked on plates; no reaction.

Aesculin broth : In 24 hrs. slight change but no black reaction; later medium darkened slowly in several days becoming black.

MacConkey's N.R.B. broth : No reduction to canary yellow in 48 hours.

Gelatine Colonies : (1st appearance), 72 hours at room temperature. Surface colonies yellowish white by transmitted light, $\frac{1}{2}$ - $1\frac{1}{2}$ mm. diameter; a characteristic depression immediately around edge of colony could be seen on tilting the plate; no bluish appearance; no liquefaction. Under the low power objective colonies pale yellow, with paler rim, and entir e edges, structure finely granular.

Agar colonies: 20 hrs. 37°C. growth slow, punctiform, scarcely visible to the eye. Examined 3 days; by transmitted light surface colonies greyish white, elliptical and round, the larger colonies .5 mm. diameter. Subsurface colonies similar to above; majority of the colonies immediately under the surface. Under the low power objective all colonies appeared dense compact with edges entire to slightly serrated.

TEMPERATURE RELATIONS :

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

Optimum temperature : On agar grows moderately well, room temperature and at 37°C.

Vitality on Culture Media : The culture survives several months in artificial medium, agar or gelatine.

RELATION TO OXYGEN : Incubated for 36 hours under anaerobic conditions scant growth on glucose agar, small gas bubbles in medium, clouding of condensation water. While growth is noted, the organism prefers aerobic conditions.

BIOCHEMICAL REACTIONS :

Indol production : Indol not produced.

Reduction of nitrates : ?

Voges Proskauer reaction : Negative.

Methyl red reaction : Slightly acid.

Fermentation of Carbohydrates : The carbohydrates used are but feebly acted upon by this culture. In each case, however, -with the exception of inulin - those substances which are fermented to gas have

shown the positive reaction within 24 hours at 37°C., and no further gas production has taken place even after 5 days. The Andrade indicator has changed to a clear scarlet and no reduction has taken place after prolonged incubation. The two substances most easily acted upon are glucose and saccharose.

Glucose.	Lactose.	Saccharose.	Mannite.	Dulcite.
+ +	+ +	+ +	+ +	+ -

Adonit.	Raffinose.	Arabinose.	Xylose.	Salicin.
- -	+ +	+ +	+ +	+ +

Aesculin.	Glycerine.	Inulin.
+ +	+ -	+ ±

\+ = acid.

++ = acid and gas.

CULTURE 32.

Source : Can I. Ser. I. Packer B.

MORPHOLOGY : Microscopically short, thick rods twice as long as broad; average length 1.6 μ staining unevenly with Kuhne's methylene blue; some longer and thinner forms, but repeated replating has failed to show impurity. Gram negative*; from old agar cultures no evidence of spores.

MOTILITY : In hanging drop occurring singly and in twos, actively motile, progression as in semicircles.

CULTURAL CHARACTERISTICS :

Agar Slope : 36 hrs. 37°C. luxuriant, raised, thick, along track of needle, glistening, iridiscent, yellowish white transmitted light, medium slight tendency to brown.

Herring agar : 20 hrs. 32°C. growth abundant and heavy along track of needle, contoured, yellowish white; spreading over slope as bluish film of discrete colonies, transmitted light, glistening, iridiscent; heavy clouding condensation water.

Loeffler's Blood Serum : 24 hrs. 37°C. moderate, moist, spreading; no liquefaction after 7 days.

Loeffler's Malachite Green Sol.: Coagulated as soft junket like curd attached to sides and bottom of tube, green, gas bubbles, light green clear fluid expressed; after 14 days coagulum as precipitation on sides of tube, no reduction of colour.

Gelatine stab : Room temperature - 24 hours filiform, no liquefaction; in 4 days growth abundant; in 1 week no liquefaction and no change in medium, growth equally good in stab and on surface; no liquefaction in 21 days.

Nutrient broth : 24 hrs. 37°C. Moderate, clouding, slight pellicle easily dislodged, pale bluish rim at top, very slight tendency to flocculency; in 48 hours flocculent precipitate suspended and at bottom; in 7 days discrete particles adhering to tube at surface, even clouding, clotted sediment on shaking.

Herring broth : similar to above, but much heavier.

Milk : 18 hours, 37°C. much froth on shaking, no coagulation; in 72 hours coagulation beginning, frothy; in 4 days weak coagulum with whey expressed, gas bubbles, curd splitting, whey white cloudy; in 10 days condition accentuated, no liquefaction.

Litmus milk : In 18hours frothy, no coagulation, violaceus, merging into light violaceus near bottom of tube; in 48 hours lilaceus, frothy, no coagulation; in 72 hours still frothy, coagulation beginning; in 4 days coagulated, some whey expressed, curd split by gas holes; in 14 days bleached with red rim at top.

Aesculin agar : 1 loop from peptone broth culture streaked on plates. In 24 hours 37°C. growth brown-black reaction.

Aesculin broth : In 24 hours, black reaction.

MacConkey's N.R.B. broth: No reduction to canary yellow in 48 hours.

Gelatine colonies : Room temperature (1st appearance), In 72 hours growth luxuriant and rapid; surface colonies up to ½ - 1 mm. diameter, white and glistening; depression around edge of colony, as if gelatine under tension - See Culture 26. Smaller colonies bluish to white, round; subsurface colonies small, bluish to bluish white. Under low power objective surface colonies dense pale yellow with paler rim and entire edges, structure finely granular; subsurface colonies similar with homogenous structure, round, edges clearly defined and entire.

Agar colonies : 20 hrs. 37°C. growth rapid, abundant, surface colonies 1½ - 2 mm. diameter, concave, smooth, glistening, tendency to striate; by transmitted light ferric to yellowish white centre, paling to blue tint at edges, smaller colonies bluish white; subsurface colonies up to .5 mm. diameter, yellowish white. Under low power objective surface colonies finely granular structure, ferric yellow paling at edges, edges entire; subsurface colonies dark "mound" appearance in centre, remainder pale lemon, finely granular with tendency to grumose, edges entire.

Herring agar colonies : Two to three times diameter of above, umbonate, radiate, concentrically ringed,

TEMPERATURE RELATIONS :

Thermal death point : Some variation has been exhibited and further tests require to be made; tests performed up to the present indicate the T.D.P. to be around 60°C. exposed for 10 minutes in nutrient broth.

Optimum temperature : Cultures incubated at room temperature and at 37oC. grow well; most satisfactory growth at 37°C.

Vitality on culture media : The cultu re survives several months on artificial medium, agar or gelatine.

RELATION TOOXYGEN : The culture is a facultative anaerobe; incubated for 36 hours at 37°C. under anaerobic conditions moderate growth on slope of glucose agar; medium cracked and split by gas bubbles, much froth in tube and heavy clouding of condensation water. The organism appears to grow equally well in the presence or in the absence of oxygen.

BIOCHEMICAL REACTIONS :

Indol production : Indol not produced.

Reduction of nitrates : Nitrates reduced to nitrites.

Voges Proskauer reaction : Negative.

Methyl red reaction : Alkaline.

Fermentation of Carbohydrates : The action of this culture on lactose is feeble and slow, gas not appearing until the second day; dulcite is but slightly fermented to acid and no gas is produced. Aesculin is fermented to acid and gas in 24 hours and in 9 days the Andrade indicator reduced to a lemon yellow turbid inidiscent colour, while no reduction is noted in the case of salicin. All the other test substances are fermented to acid and to gas rapidly with profuse frothing and heavy turbidity within 24 hours.

Glucose.	Lactose.	Saccharose.	Mannite.	Dulcite
+ +	+ +	+ +	+ +	+ -
Adonit.	**Raffinose.**	**Arabinose.**	**Xylose.**	**Salicin.**
- -	+ +	+ +	+ +	+ +
Aesculin.	**Glycerine.**	**Inulin.**		
+ +	+ +	+ +		

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

CULTURE 34.

Source : Can II, Ser' I, Packer A.

MORPHOLOGY : Microscopically varying from coccus forms to short rods; the majority .8 - 1 μ long and twice as long as broad, many thinner; stain unevenly with Kühne's methylene blue; Gram negative *; from old agar cultures no evidence of spores.

MOTILITY : In hanging drop occurring singly and in twos; actively motile.

CULTURAL CHARACTERISTICS :

Agar slope : 36 hrs at 37°C. moderate along track of needle, glistening iridiscent, bluish by transmitted light, gas bubbles in medium presumably due to fermentation of the muscle sugar in beef extract. In agar culture 2 months old distinct sliminess has been noted.

Herring agar : 20 hrs. 32°C. growth abundant, contoured, yellowish white growth along track of needle, spreading over slope as bluish film of discrete colonies; glistening, iridiscent; heavy clouding of condensation water.

Loeffler's Blood Serum: 24 hrs. 37°C. Moderate, ferric yellow, growth, no liquefaction after 7 days.

Loeffler's Malachite Green Sol.: In 24 hrs. 37°C. coagulated as described in Culture 32; in 14 days medium assuming a greenish brown tint, no definite reduction and no liquefaction.

Gelatine stab : Room temperature - 24 hours filiform, no liquefaction, equally good on surface and in stab; in 4 days growth abundant; in 7 days no liquefaction and no change in medium; no liquefaction in 21 days.

Nutrient broth : 18 hrs. 37°C. clouding moderate, slight pellicle; on shaking small flakes perceptible in medium; bluish rim; slight viscid sediment; in 72 hours cloudy waves, as watered silk, some flocculent precipitation in suspension.

Herring broth : Similar to above, but heavier.

Milk : 18 hrs. 37°C. much froth on shaking, no coagulation; in 48 hrs. weak coagulum beginning; in 72 hrs. coagulated with gas and expulsion of whey, curd later splitting with gas holes.

Litmus milk : 18 hrs. 37°C. much froth on shaking lilaceous, no coagulation; in 48 hours weak coagulation beginning; in 72 hours coagulated, gas, whey expressed, later bleaching to isabella and much splitting of curd by gas.

NOTE :- Milks and litmus milks incubated for 2 months have appeared to be slowly digesting; up to the present I have been unable to verify this and further tests must be made to establish the final condition of the clot.

Aesculin agar :1 loop from peptone broth culture streaked on plate. In 24 hours at 37°C. reaction brown-black.

Aesculin broth : In 24 hours black reaction.

MacConkey's N.R.B.Broth :In 48 hours, 37°C. slight reduction to eosin tint, but no final reduction to canary yellow.

Gelatine colonies : Room temperature (1st appearance) surface colonies up to ½ mm. diam.; by transmitted light bluish-white, glistening, almost transparent, resmelbing more the description of the B.typhosus colonies than the typical B.coli colony; flat; subsurface colonies smaller, white to yellowish-white, depression around edges, see Culture 32. Under the low power objective surface colonies pale yellow, paling near rim with edges entire; structure finely granular with a clearly defined border around more dense central structure; subsurface colonies similar.

Agar colonies :20 hrs. 37°C. growth moderate, not so rapid as other cultures; surface colonies 1 - 1½ mm. diameter, round, concave, glistening; by transmitted light bluish with pin point dark white centre, distinctly radiate. Subsurface colonies dirty white; organism growing better just under surface. Under low power objective surface colonies dark centre, remainder of colony faintly discernible as finely granular lemon yellow, with edges entire; subsurface colonies dark, compact, too dense for structure to be differentiated, edges entire.

TEMPERATURE RELATIONS :

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

Optimum temperature : Cultures incubated at room temperature and at 37°C. grow well; most satisfactory growth at 37°C.

Vitality on Culture medium : The culture survives several months on artificial medium, agar or gelatine.

RELATION TO OXYGEN : The culture is a facultative anaerobe; incubated for 36 hours at 37°C. under anaerobic conditions growth scant on slope as fine discrete colonies; heavy growth and clouding in condensation water. Slope broken and cracked by gas bubbles, these ½ cm. diameter and extending throughout the medium; much froth.

BIOCHEMICAL REACTIONS :

Indol production : Indol not produced.

Reduction of Nitrates : Nitrates reduced to nitrites.

Voges Proskauer reactions : Negative

Methyl red reaction : Alkaline.

Fermentation of Carbohydrates : The culture has a characteristic action upon dulcite; this test substance being fermented profusely to gas in 48 hours; acid and some gas produced within 24 hours. In aesculin gas appears within 48 hours. Inulin is fermented to gas only after 7 - 10 days incubation. The remaining test substances are fermented moderately well to acid and gas within 24 hours; but in no case on further incubation is the reaction profuse as in the fermentation of dulcite.

Glucose.	Lactose.	Saccharose.	Mannite.	Dulcite.
+ +	+ +	+ +	+ +	+ +

Adonit.	Raffinose.	Arabinose.	Xylose.	Salicin.
- -	+ +	+ +	+ +	+ +

Aesculin.	Glycerine.	Inulin.
+ +	+ -	+ +

\+ = acid.

++ = acid and gas.

CULTURE 35.

SOURCE : Can III, Ser. I, Packer A.

MORPHOLOGY : Microscopically large coccus forms to short thick rods; .8 mm. diam. to 1 mm. long; stain evenly with Kühne's methylene blue; Gram negative*; from old agar cultures no evidence of spores.

MOTILITY : In hanging drop appearing singly and in twos; no motility.

CULTURAL CHARACTERISTICS :

Agar Slope : 36 hrs. 37°C. Moderate to abundant along track of needle, glistening, iridiscent, porcelain white by transmitted light.

Herring Agar Slope : 20 hrs. 32°C. growth abundant, yellow-ish white along track of needle, raised edges, glistening iridiscent, by transmitted light the thinner parts bluish discrete colonies.

Loeffler's Blood Serum: 24 hrs. 37°C. luxuriant, moist; no liquefaction after 7 days.

Loeffler's Malachite Green Solution :24 hrs. 37°C. Coagulated junket-like coagulum clinging to sides of tube, gas; in 72 hours reduced greenish yellow; in 14 days reduced to yellowish-brown slimy looking liquid, partially digested.

Gelatine Stab: Room temperature, in 24 hours filiform growth equally good surface and stab, no liquefaction, slight gas - presumably from musble sugar - growth luxuriant. No liquefaction in 21 days.

Nutrient Broth : 18 hrs. 37°C. clouding even, no pellicle, no sediment, bluish rim at surface; in 48 hrs heavy clouding, viscid sediment at bottom on shaking; in 72 hours flocculent suspension, later sediment increasing, medium becoming clearer, and flocculency.

Herring Broth : Moderate clouding, bluish rim at surface, pellicle, viscid precipitate on shaking; in 4 days very heavy brown-black sediment, later flocculency and heavy clouding.

Milk : In 18 hrs. at 37°C. frothy but no coagulation In 72 hrs. coagulation commencing, gas; in 4 days gas holes in curd frothy; in 10 days clear whey on surface of soft gassy curd. In 2 months no digestion.

Litmus Milk : In 18 hours 37°lilaceous, much froth and gas, no coagulation; in 72 hours coagulation beginning; in 10 days tinted whey on surface of soft curd pinkish to isabella; no digestion in 2 months.

Aesculin agar. 37°C. One loop from peptone broth culture streaked on plates; in 24 hours reaction brown to black.

Aesculin broth. 37°C. Black reaction in 24 hours.

MacConkey's N.R.B.Broth: 37° In 48 hours no reduction to canary yellow.

Gelatine Colonies : (Room temperature) (1st appearance). Surface colonies up to ½ mm. diameter, bluish white to white, glistening, smaller colonies more distinctly blue; depression around colonies as noted Culture 32. Subsurface colonies yellowish white small. Under low power objective the centre yellowish brown dense compact surrounded by pale border ⅓ diameter of colony; edges entire clearly defined and hyaline. The differentiation of border from centre bears a close resemblance to colony of Asiatic cholera (Plate 227 Kolle & Wassermann Atlas, Tafel 10), and is not unlike Plate 45 of colon colony (Park & Williams, Path. Micro-organisms, 5th edition, Page 110). In the large surface colonies the whole structure is more homogenous. Subsurface colonies appear similar.

Agar colonies : 20 hrs. 37°C. surface colonies 1½ - 2 mm. diameter. Flat to umbonate, growth rapid, colony round, surface smooth, glistening, iridiscent. By transmitted light ferric-yellow centre paling to bluish at edge. Subsurface colonies punctiform. Under the low power objective surface colonies are dark in centre, "mound" appearance, gradually merging to pale lemon-brown colour, structure finely granular to grumose; subsurface colonies similar.

TEMPERATURE RELATIONS :

Thermal Death Point : 10 minutes exposure in nutrient broth at 60oC.

Optimum Temperature : Cultures incubated at room temperature and at 37°C. grow well; better growth at 37°C.

Vitality on Culture Media: Survives several months, in artificial media, agar or gelatine.

RELATION TO OXYGEN: Facultative anaerobe; incubated at 37°C. for 36 hours under anaerobic conditions, moderate bluish growth by transmitted light, on glucose agar; spr eading over slope as bluish film, small discrete colonies with centre more opaque. Condensation water heavily clouded; much froth; medium throughout tube riddled with gas bubbles. The organism appears to grow equally well aerobically or anaerobically.

BIOCHE MICAL REACTIONS :

Indol production	:	Indol not produced.
Production of Nitrates	:	Nitrates reduced to nitrites.
Voges Proskauer r eaction	:	Positive.
Methyl red reaction	:	Alkaline.

Fermentation of Carbohydrates : The action of the culture on dulcite is variable but it evidently is able to ferment this alconol to gas, some tests being positive, some negative; the alcohol adonit on the other hand is fermented to acid and profuse gas with frothing in 24 hours. The action on inulin is somewhat characteristic, fermentation to acid and gas with frothing in 24 hours; no other strain isolated has such pronounced effect on this test substance. Within 24 hours all the remaining carbohydrates are fermented to acid and profusely to gas with very pronounced frothing. In general this cultur e is much more active in its fermentation reaction than any of the cultures hitherto described.

Glucose.	Lactose.	Saccharose.	Mannite.	Dulcite.
+ +	+ +	+ +	+ +	+±

Adonit.	Raffinose.	Arabinose.	Xylose.	Salicin.
+ +	+ +	+ +	+ +	+ +

Aesculin.	Glycerine.	Inulin.
+ +	+ +	+ +

+ = acid.

+ + = acid and gas.

CULTURE 36.

SOURCE : Can IV, Ser. I, Packer B.

MORPHOLOGY : Microscopically varying from very short stumpy rods to forms twice as long as broad; the majority .8 to 1 long, staining unevenly with Kuhne's methylene blue; Gram negative*; from old agar culture no evidence of spores.

MOTILITY : In hanging drop occurring singly and in pairs; extremely active motility.

CULTURAL CHARACTERISTICS:

Agar slope: 36 hrs. 37°C. moderate along track of needle, glistening iridiscent, procelain to yellowish white by transmitted light.

Herring Agar slope: 20 hrs at 32oC. growth moderate slightly raised dry but glistening, some discrete colonies, by transmitted light blue to yellow.

Loeffler's Blood Serum : 24 hrs. 37oC. moderate, glistening. No liquefaction after 7 days.

Loeffler's Malachite Green Sol.: 24 hrs. 37°C. coagulated as Culture 34, much gas; in 72 hours reduced to greenish yellow. In 14 days coagulum not further reduced but precipitated on sides and bottom of tube; ferric yellow liquid expressed.

Gelatine stab: Room temperature - in 24 hours filiform growth equally good on surface and in stab; in 48 hours no liquefaction growth on surface showing, moist; in 4 days growth luxuriant; in 7 days growth becoming brown, medium slightly tinted; no liquefaction after 21 days.

Nutrient Broth : 18 hrs. 37°C. moderate even clouding, no pellicle, bluish rim at top, no sediment; in 48 hours heavy clouding watered silk appearance, later sediment noticeable; no pellicle even after 10 days.

Herring broth : Moderate growth, clouding flocculent suspension, bluish rim, no pellicle; in 48 hours brown viscid sediment precipitated; in 10 days ring on surface, very heavy flocculent growth, black sediment.

Milk : 18 hrs. 37°C. Much gas on shaking, with froth persistent, no coagulation; in 14 days weak coagulum commencing and coagulation slowly completed when examined at the end of two months.

Litmus Milk : In 18 hours no coagulation, much gas on shaking with froth persisting; violaceus merging into heliotrope; no further change in 10 days; in 14 days lilaceus, no

coagulation ; when examined 6 weeks later coagulation complete, lilaceus.

Aesculin agar : One loop from peptone broth culture streaked on plates; in 24 hours 37°C. brown to black reaction.

Aesculin broth : The typical black reaction not green after 7 days; change only to brown.

MacConkey's N.R.B.Broth : In 48 hours 37°C. an eosin tint but no reduction to canary yellow after 7 days.

Gelatine colonies : Room temperature (1st appearance) in 72 hours surface colonies small, average ½ mm. diameter, glistening flat, round; by transmitted light bluishwhite, almost transparent; characteristic ring in gelatine as noted - Culture 32.; surface colonies yellowish white, small, round. Under the low power objective surface colonies round distinctly granular and dark yellow centre, surrounded by pale border and edges entire and hyaline; on gelatine, the colonies unlike those previously described.

N.B. On referring to the notes made when this culture was originally isolated six months ago, I find that on agar the colonies were characteristically different from the colonies of cultures 32, 34, or 35. It is of interest to note that this individuality has been maintained throughout a period of this length, and in spite of having many times been subcultured on laboratory media.

Agar Colonies:

20 hours, 37°C. growth rapid; surface colonies 1 - 1½ mm. diameter; flat, glistening, iridiscent; some colonies extending as thin blue protuberances over the medium; by transmitted light colonies bluish, little darker and more opaque in centre. Subsurface colonies up to .25 mm. diameter. Under the low power objective surface colonies coarsely granular, immediate centre slightly darker and well defined; remainder same structure throughout; edges entire; subsurface colonies compact, grumose to "mound" like structure; often the surrounding medium a light ferric colour due to precipitated granules with no definite outline.

TEMPERATURE RELATIONS :

Thermal Death Point 10 minutes exposure to 60°C. in nutrient broth.

Optimum Temperature : Growth satisfactory when incubated either at room temperature or at 37°C. Most satisfactory growth at 37°C.

Vitality on Culture Media: The culture survives several months on artificial media, agar or gelatine.

RELATION TO OXYGEN : Facultative anaerobe; incubated for 36 hours at 37°C. under anaerobic conditions grows on glucose agar as pale bluish thin film along track of needle, transmitted light; spreading over slope as discrete colonies; heavy cloudy growth in condensation water; much froth in tube, gas bubbles ½ cm. diameter throughout medium. The organism grows equally well aerobically or anaerobically.

BIOCHEMICAL REACTIONS:

Indol production	:	Indol not produced.
Reduction of nitrates	:	Nitrates reduced to nitrites.
Voges Proskauer reaction	:	Positive.
Methyl red reaction	:	Alkaline.

Fermentation of Carbonydrates : The culture ferments lactose to acid, but gas is not produced until 72 hours after inoculation; the amount then is small and no increase is observed on furtner incubation; glucose, saccharose, xylose, arabinose and mannite are fermented to acid with profuse evolution of gas within 24 nours. The action upon raffinose is feeble. The Andrade indicator is rapidly decolourised in the aesculin, assuming a lemon yellow tint, such persisting; this colour is partially due to the glucoside itself.

Glucose.	Lactose.	Saccharose.	Mannite.	Dulcite.
+ +	+ +	+ +	+ +	- -
Adonite.	**Raffinose.**	**Arabinose.**	**Xylose.**	**Salicin.**
- -	+ -	+ +	+ +	- - -
Aesculin.	**Glycerine.**	**Inulin.**		
+ - -	+ -	+ -		

\+ = acid.

++ = acid and gas.

CULTURE 37.

Source : Can V, Series II, Packer B.

MORPHOLOGY : Rods, three times as long as broad; average length 1.6μ. Stain evenly, Gram negative*; from old agar cultures no evidence of spores.

MOTILITY : In hanging drop occurring singly and in twos; motile; movement varying from revolving motion to a wavelike undulating motion.

CULTURAL CHARACTERISTICS :

Agar slope : 36 hrs. 37°C. luxuriant along track of needle, raised, glistening, iridiscent, yellowish-white by transmitted light; gas bubbles in medium presumably from muscle sugar in meat extract. At times, particularly in the older cultures, agar growth decidedly slimy, drawing out on the needle. In 7 days medium lemon to brown.

Herring agar : 20 hours at 32°C. Along track of needle heavy, raised, compact, greyish white, glistening, spreading as thick blue-green veil, by transmitted light slightly iridiscent, heavy clouding of condensation water.

Loeffler's Blood Serum : 24 hrs, 37°C. luxuriant, raised, white, spreading, no liquefaction in 7 days.

Loeffler's Malachite Green Sol.: In 24 hrs. precipitated light green coagulum on sides of tube; in 48 hrs. reduction to yellow beginning in 7 days reduced to yellow and almost entirely digested.

Gelatine Stab.- Room temperature - in 24 hrs. liquefaction commencing; in 48 hours crateriform to extent of 3mm., continuing down the stab as infundibuliform; in 7 days liquefaction complete and medium sharply divided into layers; immediately below surface liquefaction appears the colour of turbid whey, in successive layers turbidity and cloudiness gradually disappearing; heavy yellow flaky precipitate at bottom.

Nutrient Broth : 18 hrs. 37°C. heavy, clouding, surface iridiscent, pellicle, bluish rim easily detached on shaking - life-belt form - medium slightly flocculent; in 4 days clouding very heavy, bluish rim; later sediment.

Herring broth : Very similar to above but heavier growth;

in 4 days heavy clouding and thick bluish white pellicle; later flocculent.

Milk : 18 hrs. cogulation commencing; in 48 hours coagulated with gas and digestion well advanced; in 10 days more than half digested, whey yellowish, heavy pellicle, soft curd; in 14 days gas bubbles still persisting, digestion proceeded, ¾ tube, the soft curd adhering to the glass, digestion not proceeding directly from surface to bottom. At a later date when the organism had been in pure culture for several months, a decided ropiness was noted, milk tubes being distinctly slimy within 24 hours after inoculation. This feature appears to have developed under cultivation and has since persisted.

Litmus Milk : in 18 nours violaceus, no coagulation; in 48 hours gas, heavy pellicle, coagulated and digestion proceeding; in 4 days a yellow digested fluid extending 2 cm. below surface, remainder violaceus; in 10 days ½ digested, remainder soft gelatinous curd; in 14 days except for tint, appearance very similar to milk as noted above.

Aesculin agar : 1 loop from peptone broth culture streaked on plates. In 24 hours black reaction.

MacConkey's N.R.B.Broth : In 24 hours heavy growth. No reduction to canary yellow. Later colour slightly changed but no definite reduction.

Gelatine Colonies : (1st appearance). In 72 hours liquefaction well advanced; individual colonies up to 3 mm. diam., round, saucer-shaped, characteristic of the organisms of the proteus group; centre of colony dark white spot .25 mm. diam., then clear space, then semi-transparent rim. Under the low power objective opaque centre merging into myceloid filaments, then clear space, and heavily clouded borders with entire edges; medium unchanged, no characteristic smell.

Agar colonies : 20 hours at 37°C. growth rapid, surface colonies concave, 1½ - 2½ mm. diameter; very slimy after repeated sub-culturing drawing out on needle 10-15 cm.; glistening; by transmitted light distinctly radiate, whole colony bluish but slightly more opaque in centre; subsurface colonies bluish to white. Under the low power objective surface colonies brownish with dark opaque centre in some, finely to coarsely granular; some colonies same structure throughout; edges entire hyaline. Subsurface colonies distinct, grumose to mound like.

TEMPERATURE RELATIONS :

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

Optimum temperature : Cultures incubated at room temperature and at 37°C. grow well. Most satisfactory growth at 37°C.

Vitality on Culture Media : The culture survives several months on artificial medium agar or gelatine.

RELATION TO OXYGEN.- The culture is a facultative anaerobe; incubated for 36 hours under anaerobic conditions moderate growth on glucose agar slope, bluish tint; very heavy clouding of condensation water; on the slope seen as discrete colonies varying from a thin bluish film to concave moist colonies 1 mm. diameter with ferric yellow centre paling towards edges. The medium riddledwith gas bubbles ½ - 1 cm. diameter, much froth in tube. This organism appears to grow equally well aerobically or anaerobically.

BIOCHEMICAL REACTIONS :

Indol production : Indol produced.

Reduction of Nitrates : Nitrates reduced to nitrites.

Voges-Proskauer reaction : Positive.

Methyl red reaction : alkaline.

Fermentation of Carbohydrates : This culture ferments lactose feebly to acid, the andrade indicator showing reduction in 48 hours, and no gas is produced. Raffinose, glycerine and inulin are fermented to acid with slight production of gas; the gas in glycerine not appearing until the second day. The remaining fermentable substances are acted upon rapidly, evolving gas profusely within 24 hours. It will be seen that of the two glucosides used, Salicin and Aesculin, the former only is fermented to gas.
In the later cultural experiments a distinct sliminess appeared in all tubes, in peptone broths with and without added sugars; a pale white rim at surface observed to be slimy after several days at 37°C.

Glucose.	Lactose.	Saccharose.	Mannite.	Dulcite.
+ +	+ -	+ +	+ +	- -
Adonit.	Raffinose.	Arabinose.	Xylose.	Salicin.
- -	+ +	+ +	+ +	+ +
Aesculin.	Glycerine.	Inulin.		
+ -	+ +	+ +		

+ = acid.
++ = acid and gas.

CULTURE 64.

SOURCE : Can VI, Ser. I, Packer C.

From this source four strains have been isolated - 64, 64a, 64b aerobically, and 64c anaerobically. The similarity of the strains in culture is such that a detailed description of each is not warranted. There are, however, certain cultural differences in 64a, 64b and 64c as compared with 64, which I have thought worthy of special mention; and these have been noted in the following description.

MORPHOLOGY : Microscopically varying from coccus forms to short thick rods; the former .8 μ diameter, the latter 1½ times as long as broad; Gram negative.* The culture has been recently isolated, and no evidence of spores has been obtained; this feature cannot at present be finally reported upon.

MOTILITY : In hanging drop occurring singly and in twos; very actively motile; meteoric flashing across the field.

CULTURAL CHARACTERISTICS:

Agar Slope: 24 hours, 37°C. moderate along track of needle, flat, slightly contoured, edges well defined, iridiscent, by transmitted light yellowish white with bluish edges.

Loeffler's Blood Serum : Moderate, no liquefaction; in 72 hours moderate, much less than Culture 65.

Loeffler's Malachite Green Sol.: 24 hours 37°C. precipitate at bottom of tube, no coagulum, liquid turbid, pea green colour; in 7 days yellowish brown turbid fluid with ferric precipitate at bottom.

Gelatine Stab.: Room temperature - 24 hours, filiform, no liquefaction; in 7 days no liquefaction, growth luxuriant, surface and in stab; yellow growth in stab.

Nutrient Broth: 24 hours 37°C. even clouding abundant, 'watered silk' appearance, no pellicle, no sediment; in 7 days clouding even, no pellicle, heavy viscid yellowish shite sediment at bottom of tube.

Milk : 24 hrs. 37°C. frothy on shaking, no coagulation; in 72 hours soft coagulum, much gas whey expressed, curd shrinking; in 7 days white turbid whey, curd shrinking and split by gas.

Litmus Milk: In 24 hours lilaceus, much froth on shaking, no coagulation; in 72 hours soft coagulum, bleached to isabella, curd perceptibly shrinking; much gas; in 7 days completely bleached with heliotrope rim at surface, depth of 2 cm. turbid tinted whey, curd rapidly disintegrating and permeated with gas holes.

N.B. This culture is violent in its action upon milk.

Aesculin agar : 1 loop peptone broth culture streaked on plates. In 24 hours 37°C. black reaction.

Aesculin broth : In 24 hours, 37°C. black reaction.

MacConkey's N.R.B.Broth: In 48 hours, 37°C. reduced to canary yellow.

Gelatine Colonies: Room temperature (1st appearance) - identical with Culture 35 - 64a presents some variation. In 72 hours growth rapid abundant, more luxuriant than any of foregoing cultures; surface colonies up to 1 mm. diameter, compact, white, opaque with tendency to capitate, round; the smaller colonies bluish to bluish white. Subsurface colonies small compact. Under the low power objective surface colonies have appearance identical with the literature description of the B.coli colony, edges entire centre dark and opaque; subsurface colonies pale yellow in colour, very finely granular, slightly darker in centre. See Culture 35.

Agar Colonies : 20 hours, 37°C. growth rapid, flat, surface colonies 1½-2½ mm. diameter, round with tendency to spread; by transmitted light distinct bluish appearance, glistening, iridiscent. Subsurface colonies up to .25 mm. diameter bluish to white. Under the low power objective surface colonies have small well defined dark centre, remainder lemon coloured; structure coarsely granular to grumose, edges entire, hyaline and well defined; pale radiate filaments - star-like rays - emanate from the colonies into surrounding medium. Subsurface colonies dark grumose to "mound" like.

Agar Colonies 64a. 20 hours 37°C. growth rapid, surface colonies bluish from 1 - 2 mm. diameter, glistening, iridiscent, tendency to run together, forming blue film over agar. Subsurface colonies up to 1 mm. diameter white to yellowish white; some force their way to surface and appear as yellowish-white in centre, spreading on surface to 3 mm. diameter, blue, flat, concentrically ringed, contoured, edges undulate to lobate. Under the low power objective surface colonies (majority) finely granular at centre to grumose near edge; in some instances characteristic protuberances over agar as in Culture 64, edges entire; subsurface lemon yellow, edges entire.

TEMPERATURE RELATIONS :

Thermal Death Point : Exposed in nutrient broth for 10 minutes at 60°C. organism survives; exposed for 10 minutes at 70°C. no subsequent growth; exact temperature not yet definitely determined.

Optimum Temperature : Grows well at room temperature and at 37°C. More satisfactory growth at 37°C.

Vitality on Culture Media : Not yet determined.

RELATION TO OXYGEN : The culture is a facultative anaerobe; incubated for 36 hours under anaerobic conditions at 37°C. the medium -- glucose agar -- is split, riddled with gas bubbles and upper portions blown to top of tube, much froth; heavy cloudy condensation water permeated whole of medium. The organism grows with extreme rapidity both aerobically and anaerobically.

CHEMICAL REACTIONS :

Indol production : Indol not produced.

Reduction of Nitrates : Nitrates reduced to nitrites.

Voges Proskauer reactions : Positive.

Methyl red reaction : Alkaline.

Fermentation of Carbohydrates : The culture fails to ferment dulcite and adonit to acid or gas. All other test substances used are fermented within 24 hours to acid, and profusely with much frothing to gas. In the glucose, lactose, saccharose, mannite, raffinose, and arabinose tubes the Andrade indicator is completely reduced within 24 hours, the reduction in the xylose, salicin and aesculin tubes being slower. Compared with the other cultures described herein the rapid and violent action upon the carbohydrates is both distinctive and characteristic, as also is the rapidity with which the Andrade indicator is decolourised. The decolourised tubes when tested with methyl red showed decided alkalinity. The rapid reversion to an alkaline reaction is a point of considerable interest.

64a. The fermentation reactions are identical with those of the above culture, but a striking difference, which may be but temporary however, has been noted in the action upon the Andrade indicator. No reduction of the indicator in any tubes was noted within 24 hours; in 72 hours glucose, mannite, arabinose, xylose and salicin had changed from the scarlet tint of the acid reaction to a deep pink shade. In 7 days the glucose, arabinose, and xylose tubes only were completely reduced giving an alkaline reaction to methyl red.

I have as yet no explanation to offer regarding this apparent selective action towards the Andrade indicator; the inoculations were made at the same time, the same amount of the respective peptone broth cultures being added as the inoculum, such broth cultures being the same age, and all medium used of the same standard stock.

In this connection it may be of interest to mention that for some months I have been experimenting with Congo red as an indicator in connection with routine water analyses for the colon group; these experiments are as yet not sufficiently complete for publication; I have used this indicator in sugar broths as a confirmatory test and find that the strains 64 and 64a exhibit again, as in the Andrade indicator, a selective action.

Glucose.	Lactose.	Saccharose.	Mannite.	Dulcite
+ +	+ +	+ +	+ +	- -
Adonit.	**Raffinose.**	**Arabinose.**	**Xylose.**	**Salicin.**
- -	+ +	+ +	+ +	+ +
Aesculin.	**Glycerine.**	**Inulin.**		
+ +	+ ±	+ +		

\+ = acid.

++ = acid and gas.

Culture.	Morphology	Gram's Stain	Spores.	Motility	Agar slope	Blood Serum	Malachite Green Sol.	Gelatine Stab.	Nutrient Broth.	Milk.	Aesculin Agar.	MacConkey's N.R.B. broth	Thermal Death pt.	Optimum Temperature	Relation to Oxygen.	Source.
24.	Coc. to rod	-	-	+	Lux.	Mod. No liq.	Coag. part. dig.	Liq.	Cloud. heavy	[a] Coag. 72hr. dig.	Brown black	No reduc.	C. 60°	C. 37°	Facul. anaer. prefers aerob.	Can II Ser.II Packer A.
[b] 37	Rod	-	-	+ Wave like	Lux.	Lux. No liq.	Reduced.	Liq.	Cloud. heavy pell.	Coag. 18hr dig. slimy	Black	Slight redc.	60°	37°	facul. anaer.	Can V, Ser.I. Packer B.
32	Rod	-	-	[c] + Act.	Lux.	Mod. no liq.	Coag. no reduc.	No liq.	Mod. cldg. pell.	Coag. 72 hrs.	Brown black	No reduc.	[d] 60°	37°	fac. anaer.	Can I, Ser.I, Packer B.
36	Rod	-	-	+ act.	Mod.	Mod. no liq.	Coag. slgh. reduc.	No liq.	Mod. no pell.	Coag. 14 days.	Brown black	No reduc.	60°	37°	fac. anaer.	Can IV, Ser.I, Packer B.
26	Rod	-	-	-	Mod.	Slt. no liq.	Coag. no reduc.	No liq.	Mod. cloud.	Coag. 72 hrs.	[e] No reaction	No reduc.	60°	37° & 21°	facul. anaer. prefers aerobic	Can III, Ser.II, Packer A.
34	Coc. to rod	-	-	+ act.	Mod.	Mod. no liq.	Coag. not reduc.	No liq.	Mod. cloud	Coag. 48 hrs.	Black	No reduc.	60°	37°	facul. anaer.	Can II, Ser.I, Packer A.
35	Coc. to rod	-	-	-	Mod. to lux.	Lux. no liq.	Coag. reduc.	No liq.	Even cloud, no pel	Coag. 72nrs No dig.	Brown to Black	No reduc.	60°	37°	facul. anaer.	Can III, Ser.I, Packer A.
64	Coc. to rod	-	-	+ very act.	Mod.	Mod. no liq.	Precip. Reduced.	No liq.	Abun. cloud	Coag. 72 hrs.	Black	Reduc. to canary yellow	[f] 60° to 70°	37°	Facul. anaer.	Can VI, Ser.I, Packer C.

a sliminess noted as digestion proceeds.
b sliminess appearing after successive subculturing.
c Motion as in semi
d see page
e Aesculin fermented to gas but no action on aesculin agar.

Culture.	Indol.	Nitrate Reduction.	Voges Proskauer.	Methyl Red.	Glucose.	Lactose.	Saccharose.	Mannite.	Dulcite.	Adonite.	Raffinose.	Arabinose.	Xylose.	Salicin.	Aesculin.	Glycerine.	Inulin.	Source.
24.	-	+	+	Alk.	++	+-	++	++	+-	—	++	++	++	++	++	++ g	++	Can II. Ser.II Packer A.
37	h +	+	+	Alk.	++	+-	++	++	—	—	++	++	++	++ i	+-	++	++	Can V. Ser.I Packer B.
32	-	+	-	Alk.	++	++ k	++	++	+-	—	++	++	++	++	++ L	++	++	Can I. Ser.I. Packer B.
36	-	+	+	Alk.	++	++ m	++	++	—	—	+-	++	++	—	+- o	+-	+-	Can IV. Ser.I. Packer B.
26	p -	?	-	Acid	++	++	++	++	+-	—	++	++	++	++	++ e	+-	+- +	Can III. Ser.II Packer B.
34	-	+	-	Alk.	++	++	++	++	++ q	—	++	++	++	++	++	+-	++ r	Can III. Ser.I Packer A.
35	s -	+	+	Alk.	++	++	++	++	+- +	++	++	++	++	++	++	++	++ t	Can III. Ser.I. Packer A.
64	x -	+	+	Alk.	++	++	++	++	—	—	++	++	++	++	++	+- +	++	Can VI. Ser.1. Packer C.

\+ acid.
++ acid & gas.
g gas after 72 hrs.
h sliminess appearing after successive subculturing.
L Note production of gas in one glucoside, not in the other.
k feebly to gas.
l reduced, lemon yellow
m. gas not produced until 72 hrs.
o Andrade indicator rapidly decolourised.
q characteristically profuse.
r reaction very slow
s pronounced and rapid action upon carbohydrates.
t action on inulin pronounced, more so than any of the series.
x the carbohydrates attacked are fermented profusely to gas within 24 hours; rapid reduction of Andrade indicator and characteristically rapid reversion to alkalinity.

EXPERIMENTAL SWELLED CANS.

Having isolated strains of gas-producing bacteria from swelled cans of sardines, and having determined their cultural features and biochemical reactions, the next step was to attempt the experimental swelling of normal cans by inoculation of organisms already isolated. Up to the present I have used three cultures for this purpose - Cultures 35, 37 and 64. These three cultures on the basis of their biological and biochemical reactions are sufficiently differentiated (Page) to warrant individual trials. A number of normal cans of sardines were most courteously supplied by the Manager of the Chamcook factory, St. Andrews, N.B. Some of the cans were of sardines packed in cotton-seed oil, olive oil having been used for the remainder. The cans had to be 'punched', inoculated, and again sealed. In order to eliminate as far as possible any error of manipulation I obtained by courtesy of the Chief Engineer the services of the College plumber, who undertook the soldering. To avoid trouble from escaping oil, the cans were placed on end rather than flat on the bottom. By the usual method a layer of solder was first spread over a portion of the can; this I cleaned and sterilised with absolute alcohol, and then with a sterile awl punched a hole 3 mm. diameter. From a 1cc. pipette 2 - 3 drops of a young peptone broth culture of the desired organism were quickly dropped in; a small square of sterilised tin heated in the flame was at once placed over the hole and the soldering process performed. The layer of solder previously spread over the can assisted materially in making the process effective. In this manner, cans were inoculated with the respective cultures; the control cans receiving exactly the same

treatment minus the inoculation. The cans, each placed in the half of a large petri dish, were incubated at a temperature of 30°- 33°C. They were examined at frequent intervals, and in 4 days swelling was observed in those inoculated. In 7 days the swelling had become so pronounced that there appeared to be danger from explosions. The cans were examined.

NORMAL CANS.

(Punched and resoldered). These appeared perfectly normal; no oil in petri dish, no moisture on outside of can, no swelling, no 'rattle' on shaking. When opened there was no escape of gas; contents firm in texture, flesh the white of the normal sardines, and comparatively dry; odour typical and mild; normal in every respect.

Can 35 : Inoculated with Culture 35; oil in petri dish and on surface of can; pronounced swelling, top and bottom of can convex; on shaking, the typical 'rattle' of the original 'swells'; when opened, escape of gas and exuding of oil. The contents were soft, moist, and disintegrated to an even greater degree than in many of the original 'swells.' The oil was intermixed with the macerated sardines and gas bubbles were very evident throughout the whole. The colour was a little darker than normal. The odour was not putrefactive, but an accentuation of the typical normal smell. The conditions noted were as evident on the side immediately opposite the point of inoculation, as at the point of inoculation itself. The condition of this can and its contents was in every respect identical with the conditions found when examining the original typical 'swells.'

Can 37 : Inoculated with Culture 37 - the description given of Can 35 is here strictly applicable; no variation could be noted.

Can 64 : Inoculated with Culture 64. The swelling of this can was more pronounced, otherwise the description given of Can 35 is here strictly applicable in every respect.

ISOLATION OF ORGANISMS.

Pieces of fish were taken from the respective cans and inoculated into series of liquid media; glucose peptone broth, peptone broth, and nutrient broth respectively. These tubes were incubated at 37°C. for 24 hours. Pronounced clouding of the media by each inoculum was by that time evident. Plates were made on glucose agar, and after incubation at 37°C. for 24 hours, typical colonies were picked off and streaked on agar slopes. Subsequently, series of inoculations were made and the respective organisms isolated proved to be identical with the strain with which the experimental cans were inoculated.

Cultures 35, 37 and 64 respectively, have experimentally produced typical swelled cans, have been re-isolated, and proved culturally identical with the original strain. The Postulates of Koch have been satisfied.

CULTURE 7.

Source : Herring Excreta.

MORPHOLOGY : Spore forming rods, occurring singly, in twos and in long forms. Gram negative.

CULTURAL CHARACTERISTICS.

Nutrient broth : In 24 hrs at 37°C. membranous pellicle, medium clear; 1 month yellow sediment, medium clear.

Milk : In 5 days pellicle, no change; in 1 month yellow turbid digestion extending 2/3 down tube.

Litmus Milk : In 24 hrs. no change; in 10 days pellicle, sediment, digestion with colour varying from yellow to dark purple.

Gelatine Stab : Room temperature, liquefaction beginning in 2 days. In 5 days napiform to a depth of 5 mm., remainder filiform; in 14 days liquefaction still proceeding with lower part of stab a discrete villous growth; medium ferric lemon.

BIOCHEMICAL REACTIONS :

Indol	not produced.
Nitrates	not reduced.
Glucose broth	acid, even clouding, no gas.

This culture in its reactions is typical of many strains isolated from herring excreta.

CULTURE 21.

Source : Normal can, Sardines, Packer A.

MORPHOLOGY : Extremely long thin rods, forming spores; in hanging drop occurring singly and in twos, motile, gram positive.

CULTURAL CHARACTERISTICS :

Nutrient broth : In 24 hrs at 37°C. slight clouding, no pellicle; in three days membranous cup-shaped pellicle, medium cloudy; later, pellicle luxuriant, thick creamy, medium yellowish brown.

Milk : No change up to 5 days, when weak coagulum beginning; in 9 days tubes half coagulated; in 16 days yellow digestion nearly complete, remainder of medium firm hard curd.

Litmus Milk : No change in 24 hours; in 3 days pellicle, upper layers of milk dark purple, remainder violaceus, no coagulation; in 9 days digested without previous coagulation to muddy looking yellowish brown liquid.

Loeffler's Blood Serum : Rapid liquefaction.

Gelatine Stab : Room temperature, in 24 hrs crateriform liquefaction beginning; proceeding clowly in 7 days to .5 cm. from surface of stab; in 18days not complete, layers of yellowish precipitate.

BIOCHEMICAL REACTIONS :

Indol	not produced.
Nitrates	not reduced.
Glucose broth	acid, chiefly at surface, no gas.

From the same can, and other normal cans, strains were isolated which according to the reactions noted, proved to be identical with this culture.

CULTURE 13.

Source : Swelled Can I Ser. II, Packer A.

MORPHOLOGY : Large coccus, occurring as staphylococcus, no spores, Gram positive.

CULTURAL CHARACTERISTICS :

Nutrient broth : 24 hrs. at 37°C. moderate, cloudy; no pellicle.

Milk : In 5 days no change; no change in 1 month.

Litmus milk : As milk.

Gelatine Stab : Room temperature. In 2 days no liquefaction; in 5 days scant growth filiform to discrete; in 14 days medium faintly browned, frowth in stab discrete and ferric yellow tint; no liqu efaction, growth better under surface.

BIOCHEMICAL REACTIONS :

Indol — Not produced.

Nitrates — ?

Glucose broth — Acid, even clouding, no gas.

CULTURE 28.

Source : Same Can as Culture 13.

MORPHOLOGY : Long rods many times longer than broad, oval spores formed; Gram negative; in hanging drop appear singly; in twos and in long chains; motile with gliding movement.

CULTURAL CHARACTERISTICS :

Nutrient Broth : 24 hrs. 37°C. Moderate, cloudy, slight pellicle; in 1 month cloudy with flocculent yellow sediment.

Milk : In 5 days no change; in 1 month digested completely, yellow turbid fluid.

Litmus Milk : In 10 days dark purple fluid with no previous coagulation; unchanged in 1 mth

Loeffler's Blood Serum : Rapid liquefaction.

Gelatine Stab : Room temperature, in 2 days slight liquefaction noted; in 5 days liquefaction progressed to dppth of 2 mm., stratiform, remainder of stab discrete colonies; in 14 days liquefaction 1 cm. depth, stratiform yellowish layers.

BIOCHEMICAL REACTIONS :

Indol	not produced.
Nitrates	not reduced.
Glucose broth	Acid, upper part, pellicle, no gas.

Cultures 13 and 28 typical of several strains isolated from such cans.

CULTURE L4.

Source : Swelled Can II, Ser. II, Packer A.

MORPHOLOGY : Long rods manytimes longer than broad; spores. Gram positive; in hanging drop occurring singly, in twos, and in chains; appear at first immobile but prolonged examination reveals slow laboured movement in some individuals appearing to push themselves along.

CULTURAL CHARACTERISTICS :

Nutrient broth : 24 hrs at 37°C. fine precipitate suspend-ed near surface; lower portion clearer, no pellicle; in 10 days even clouding throughout, with "dusty" appearance; in 1 month slight clouding, sediment at bottom.

Milk : No change in 1 month.

Litmus Milk : In 24 hrs bleached, otherwise no change; in 1 month yellowish liquid.

Gelatine Stab : Room temperature, in 2 days no liquefaction; in 5 days luxuriant growth on surface and aborescent growth in stab, no liquefaction; in 14 days no further change; liquefaction observed in 1 month

BIOCHEMICAL REACTIO NS :

Indol	not produced.
Nitrates	not reduced.
Glucose broth	acid, more particularly near surface, no gas, no pellicle.

CULTURE 16.

Source : Swelled Can III, Ser. II, Packer A.

MORPHOLOGY : Coccus, occurring also as staphylococci; .5 mm. average diame ter, no evidence of spores, Gram positive; in hanging drop many tetrads noted; non-motile.

CULTURAL CHARACTERISTICS :

Nutrient broth :	24 hrs, 37°C. cloudy, flocculent pellicle; in10 days heavy clouding with some flocculency; in 1 month clouding and yellow precipitate at bottom of tube.
Milk :	In 5 days no coagulation, ring, pellicle; in 1 month coagulated, some yellow whey expressed.
Litmus milk :	In 24 hrs no change; in 10 days lilac, no coagulation; in 1 month coagulation, and some whey expressed.
Gelatine Stab :	Room temperature - in 2 days moderate growth, dip in gelatine; in 5 days crateriform liquefaction and spreading growth on surface of stab; in 14 days liquefaction varied from V.shaped to crateriform to depth of 1 cm., cloudy; remainder of stab discrete.

BIOCHEMICAL REACTIONSv:

Indol	not produced.
Nitrates	not reduced.
Glucose broth	acid, more particularly near the surrace, no gas.

Culture.	Morphology.	Spore formation.	Motility.	Gram's stain.	Nutrient broth.	Milk.	Litmus Milk.	Loeffler's Blood serum.	Gelatine stab.	Indol.	Reduction of Nitrates.	Glucose broth.	Source.
7	Rod	+	?	-	Pellicle, med.clear	Slow digest.	Digest:	..	Liquef.	-	-	+ -	Herring excreta.
21	Rod	+	+	+	Clouding, later pellicle	Slow coag. later dig.	Digest. slowly without previous coagln.	Rapid liquef.	Liquef. slow.	-	-	+ -	Sardines, Packer A, Normal can.
13	Coccus	-	?	+	Moder. cloudy	No change	No change	...	No liquef.	-	?	+ -	Swelled Can I, Ser.II, PackerA.
28	Rod	+	+	-	Cloudy, later pellicle	Slow diges.	Cleared with no coag.	Rapid liquef.	Liquef. slowly	-	-	+ -	As Culture 13.
14	Rod	+	+ reeb.	+	Cloudy	No change	Slow digest.	...	Liquef. after 14 days	-	-	+ -	Swelled Can II (Ser.II) Packer A.
16	Coccus	-	-	+	Cloud., Later Precip.	Coag. slow	Coag. slow	...	Liquef. slowly	-	-	+-	Swelled Can III (Ser.II) Packer A.

Brief Summary

1. Forty cans of Sardines, normal, and 'Swelled' have been submitted to a bacteriological examination

2. Cotton-seed oil, and the excreta of fresh herrings have been examined

3. From the 'Swelled' cans, eight strains of gas-producing bacteria have been isolated; numbers 24, 26, 32, 34, 35, 36, 37 and 64

4. The eight strains have been studied morphologically, biologically, and biochemically; and have been described.

pages 29-53

a. Two strains, numbers 24 and 37 fail to ferment lactose; they are tentatively placed in the Proteus group

B. vulgaris (Hauser 1885)
Migula 1900

b. The remaining six strains are lactose-fermenting types; some very feebly, some profusely. ~~I do~~ I consider that these include typical and a-typical forms

of the colon-aerogenes group (Escherich); but prefer at present to offer no opinion as to their individual classification.

5 The features and the reactions of the gas-producing bacteria have been summarised on pages 54 + 55

6 Experimentally, 'swellings' in normal cans of sardines have been produced on inoculation with cultures 35, 37, and 64 respectively; and the organisms isolated found to be identical with (respectively) those used for inoculation; thus satisfying the Postulates of Koch.

7 No bacteria have been isolated from the cotton-seed oil. Non-gas-producing bacteria have been isolated from herring excreta, from 'swelled' tins, and from a small percentage of the normal cans examined. — see pgs 59-64.

8 No gas-producing bacteria have been isolated from the normal cans.

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