An Investigation of the Water Supply of the City of Vancouver, B.C.

by

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AN INVESTIGATION OF THE WATER SUPPLY OF THE CITY OF VANCOUVER

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For some years, the water supply of the City of Vancouver has been under supervision by the Medical Health Officer. Bacterial counts are made weekly of three distinct samples; one sample from Seymour water supply, one from Capilano water supply, and one taken from a faucet in the city constituting a mixture of the two. Occasionally in this weekly routine examination, organisms giving the presumptive test for Bact. coli have been found. During the past four years however, samples which were positive to the presumptive test have been subjected to a more detailed examination and in no case has it been possible to identify the organisms isolated as typical strains of Bact. coli.

The present investigation was undertaken with four objects in view:

1. To determine if possible the frequency of occurrence of organisms giving the presumptive coli test.
2. To determine if possible the source of such organisms.
3. To record what daily variations, if any, occur in the bacterial content of the water supply.
4. To determine the regular Bacterial Flora of the water supply.

The writer is indebted to the Medical Health Officer, Dr. F. T. Underhill, for much assistance, and for the privilege of consulting the information regarding the data in his

office; and is indebted also to Mr. E. M. LeFluffy of the City Engineer's office for considerable data regarding the physical condition of the water supply.

G E N E R A L D A T A.

DESCRIPTION OF THE WATER SUPPLY.

Seymour and Capilano Creeks are the sources of the water supply for the City of Vancouver. They are separate and independent of each other. Capilano watershed has a drainage area of about 55 square miles, while that of Seymour is about 80 square miles. They are both rapid, clear streams, mainly glacial water, with a fall of about 70 feet per mile. The discharge varies within wide limits, that of Capilano for instance varying from about 15,000 cu. ft. per second when in flood, down to about 40 cu. ft. per second in very dry weather (August). With the exception of a few days in each year during flood conditions the water is clear and free from sediment and does not require filtration.

INTAKES, TANKS AND SCREENS.

Capilano intake is situated 7 miles from the mouth of the Creek at an elevation of about 480 feet above sea level and 10 miles from the centre of the City of Vancouver. The intake is constructed of reinforced concrete with head racks of oak to keep out leaves and sticks. An open conduit or flume 570 feet long connects the intake with the tanks, the water being

admitted through two sluice valves. The tanks are in duplicate, each being 100 feet long by 20 feet wide. At low water they are filled to a height of 6 feet. Both are provided with a double row of screens at the mouth of the supply pipe to prevent from entering any gross suspended or floating matter.

The Seymour supply has two intakes, situated about 7 and 7½ miles respectively from the creek's mouth at elevations of 465 and 485 feet above sea level. The lower intake is similar in general design to the Capilano intake described above. The upper one is constructed of hewn cedar with rock filling. Oak racks are provided at the entrance and a conduit extends 300 feet to the settling tanks. These are in duplicate, measure 100'X20'x6', and are lined with concrete. Double rows of screens are provided at the lower end of each tank.

DISTRIBUTION SYSTEM.

The water from both intakes flows into 36 inch mains, the Capilano supply crossing the Inlet at the First Narrows and the Seymour supply at the Second narrows. Just before crossing, the 36 inch pipes are divided into four 16 inch pipes, one of which goes directly to Burnaby, while the others supply the remaining Municipalities. The supply from Seymour and Capilano is not distributed as such to different parts of the city, but in some portions, especially in the West End, there are cross connections between these two supplies in order to ensure water in case any one pipe under the Narrows should be destroyed.

RESERVOIRS.

The water flows by gravity directly into the mains but in addition there are two reservoirs known respectively as the "Stanley Park" and "Little Mountain" reservoirs. The Stanley Park reservoir, with a capacity of about ten million gallons, is situated 240 feet above the sea level. It is now used only as a balancing and emergency reservoir since its elevation is not sufficient to give adequate pressure throughout the whole city. The Little Mountain reservoir, distant about one mile south of the city boundary, has an elevation of approximately 400 feet above sea level, with a capacity of twenty-five million gallons. This reservoir is filled and maintained from the Seymour Creek supply main. It is constructed of concrete and controls the pressure over the whole city.

SERVICES.

The approximate number of services for house, office, and factory connections in the City of Vancouver is 25,000. This does not include the other municipalities served. The pipes vary in size from ¾" to 6" in diameter, the majority of the house connections or domestic services being ¾" in diameter.

CONSUMPTION.

The average amount of water admitted at the intakes totals thirty million gallons per day. This does not represent the consumption of Vancouver proper, as at the present time the

Municipalities of Point Grey, South Vancouver and Burnaby are also supplied with water from the city mains.

The 1920 particulars as to population and consumption are as follows:-

Location.	Population.	Consumption. (in million gals. a day.)
City of Vancouver,---	125,000	25
Point Grey,----------	17,000	1.5
South Vancouver,-----	30,000	1.9
Burnaby,-------------	17,000	1.6
	189,000	30.0

L A B O R A T O R Y S T U D I E S.

1. QUANTITATIVE DETERMINATIONS.

Samples of tap water were examined daily, Sundays and one or two other days excepted, from November 23rd, 1920 to May 6th, 1921, inclusive. Direct counts and the presumptive test for Bact. coli were made as a routine preliminary procedure each day.

Media Employed.

Beef agar, gelatin and lactose broth made according to Standard Methods (1920) (1) were used. The reaction was adjusted so that the final reaction was PH 7. Brom-thymol blue was used as the indicator in adjusting this reaction and as indicator in the lactose broth.

Technique.

The mouth of the tap was sterilized by flaming for 3 minutes after which the water was allowed to run for 5 minutes. About 50 cc were then collected into a sterile flask. 1 cc and 0.1 cc amounts were plated on agar and gelatin, and the plates incubated at 37°C and room temperature respectively. For the presumptive test 1 cc of the water was inoculated into lactose broth fermentation tubes. The agar plates were counted after 24 hours incubation at 37°C and the gelatin after 3 days at room temperature. The results of the presumptive tests were recorded

after incubation at 37°C for 48 hours. The production of acid and 10% or more gas at the end of this period was regarded as a positive test. The counts and the correlation of the same with heights of water at both Capilano and Seymour intakes are graphed in Table I.

The points in each curve are joined by a continuous line. The substitution of a dotted line indicates the occurrence of "spreaders" on the plates preventing counts.

The bacterial count at 37°C varies from 0 per cc to 12 per cc. The range at 22°C is from 29 per cc to 105 per cc. It is interesting to note that there appears to be a correlation between both the 37°C counts and the 22°C counts, and the height of the water at the intakes. This is close enough to be significant. Undoubtedly, a higher count is to be expected after a period of high water at the intake when a certain number of soil bacteria must have been washed into the supplies by rains or melted snow.

On three days only were presumptive tests positive, namely, December 2nd, December 10th, and December 11th. There does not seem to be correlation between the occurrence of these presumptive tests and the height of the water at the intakes, but there is an apparent relation between the occurrence of the presumptive test and the rainfall. At least it may be stated that on the three occasions upon which the presumptive tests were positive there had been an immediately preceding heavy rainfall, as is shown by Table II.

TABLE I

100
90
80
70
60
50
40
30
20
10

... NO. OF BACTERIA PER cc AT 22°C

.... HEIGHT OF WATER AT CAPILANO INTAKE IN INCHES

HEIGHT OF WATER AT SEYMOUR INTAKE IN INCHES

.....NO. OF BACTERIA PER cc AT 37°C

Nov 23 24 25 26 30
Dec. 2 3 4 6 7 8 10 11 12 13 14 15 17 18 19 20 21 22 23 24 25 26 27 28 29 31
Jan. 1 2 3 4 5 7 8 9 10 11 12 14 15 16 17 18 19 21 22 23 24 25 26 28
Feb. 2 3 4 5 7 8 9 10 11 12 14 15 16 17 18 19 21 22 23 24 25 26 28
Mar. 2 3 4 5 7 8 9 10 11 12 14 15 16 17 18 19 21 24 25 26 28 29 30 31
April 1 2 4 5 6 8 9 11 12 13 14 15 16 18 19 20 21 22 23 25 26 27 28 3 4 5 6
May

TABLE II.

Showing relationship between rainfall and positive presumptive test.

Date	December 1	2	3	4	5	6	7	8	9	10	11	12	&
Capilano	.07	1.66	.85	.70	.03	.34	1.54	1.03	.17	1.86	3.6	.10	
Seymour	1.32	.98	.98	.05	.56	.97	.68	.45	1.33	2.40	.08	1.63	
Presumptive Test	0	+	0	0	0	0	0	0	0	+	+	0	

2. QUALITATIVE DETERMINATIONS.

Media employed and methods adopted.

The following media made according to the Standard Methods of Water Analysis (1920) (1) were used. Agar, broth, gelatin, peptone, phosphate broth for Methyl Red test, sugar broths and endo-agar. Potato medium was made in the ordinary way. The milk media are described later (see pages 15 & 16). Neutral red bile salt lactose agar (4) was used in search for lactose-fermenting organisms. Erhlich's Indol test, (3) was used.

The morphology is described on 24 hour agar cultures incubated at 37°C, unless otherwise stated. Stirling's modification of Gram stain was used; Staphylococcus aureus and Bact. coli being employed as positive and negative controls in each case. In determining liquefaction of gelatin, cultures were kept under observation for eight weeks before a negative liquefier was recorded.

Types of organisms found in Daily Counts.

Colonies for investigation were fished from both agar and gelatin plates. In selecting bacteria for study it soon became a matter of comparative ease to pick off typical colonies of various types. On the three occasions on which the presumptive test was positive, lactose plates were made from the fermentation tubes and from them, lactose fermenting colonies picked. The following five distinct types of organisms

were obtained:

1. Sarcinae and Rhodococci.
2. Spore formers.
3. Rapid lactose-fermenters.
4. Slow lactose-fermenters.
5. Fluorescent non-lactose fermenters.

TYPE 1. SARCINAE AND RHODOCOCCI.

Several strains of the genus sarcinae were commonly found on the agar and gelatin plates.

General Characters.

These bacteria are spherical in shape, and divide under favorable conditions in three planes producing packet groups of eight.

In these cultural studies growth was abundant on artificial media, pigment being formed on agar, gelatin and potato. Gelatin was usually liquefied.

ORGANISM NO. (1).

The morphology was determined on beef peptone agar after 48 hours incubated at 22°C. The organism occurs as large spherical cocci 1.5 to 2 mierons in diameter, arranged in packets of eight, non motile. Optimum temperature 22°C. Slightly decolourized by Gram stain.

Cultural Characters.

Agar slant:- growth abundant, lemon yellow, echinulate, raised.

Broth:- heavy clouding, yellow sediment.

Gelatin:- abundant growth along line of puncture. Slow napiform liquefaction.

Carbohydrates:- dextrose broth faintly acid, alkaline in lactose and sucrose broths.
This organism is classified after Winslow as Sarcina flava (De Bary) (5).

ORGANISM NO. (2)

Similar to S. flava except that the pigment produced is a brownish yellow.

This organism is classified after Winslow as Sarcina canescens (Stubenrath) (5).

ORGANISM NO. (3)

Morphologically identical with S. flava except that it is Gram positive.

Cultural Characters.

Agar:- abundant golden yellow growth.

Gelatin:- rapidly liquefied, a heavy orange precipitate.
Organism No. 3 is classified as Sarcina aurantiaca (Flugge) after Winslow (5).

ORGANISM NO. (4)

Morphology.

Large spherical cells occurring in packets. Gram negative. Optimum temperature 22°C.

Cultural Characters.

Agar:- abundant surface growth.

Gelatin:- pink growth along the line of puncture, very slowly liquefied.

Carbohydrates:- dextrose broth faintly acid, lactose and sucrose broths alkaline.
Classified as Rhodococcus roseus (Flugge) after Winslow (5).(6).

TYPE 11. SPORE FORMERS.

Several strains of the mycoides, cereus, subtilis and megatherium groups were commonly found on the room temperature plates, as follows (using Ford's Classification) (7).

ORGANISM NO. (5).

Morphology.

In young cultures on plain agar the organisms are long and thin and tend to arrange themselves in short chains. Motile. Gram positive. The spores appear after about 48 hours; at first they are situated at one end of the cell, but soon the cytoplasm disintegrates, leaving an oval spore. These spores often remain attached in long chains.

Cultural Characters.

On solid media the growth is glistening and rhizoid. Agar colonies are characteristic; from the central nucleii filamentous strands of growth extend on all sides forming the typical "myceloid" colony.

Gelatin:- is slowly liquefied.

Carbohydrates:- slight acidity is produced in dextrose broth and alkali in lactose and sucrose.
Classified as Bacillus mycoides (Flugge) (Ford) (7).

ORGANISM NO. (6)

This bacillus is a rapid "spreader" and was usually found after a heavy rain.

Morphology.

Small rods with rounded ends, often growing in short

chains and in old cultures, showing a tendency to thread. Actively motile. Gram positive, sometimes slightly decolourized. The spores appear early, and have the cytoplasm attached to either end.

Cultural Characters.

Agar slant:- the growth is abundant, spreading and glistening. A characteristic green pigment is diffused throughout the medium.

Agar colonies:- spreading and amoeboid with slightly raised edges and the usual green colouration.

Gelatin:- rapid saccate liquefaction. The medium soon acquired the green fluorescence.

Litmus Milk:- peptonized without coagulation. A clear greenish ring starts at the top and rapidly proceeds downard.

Carbohydrates:- dextrose is fermented to acid, lactose and sucrose unchanged. Classified as Bacillus cereus fluorescens (Ford)(7).

ORGANISM NO.(7)

Morphology.

Small square ended rods. Motility doubtful. Gram positive.

Cultural Characters.

Agar stab:- thin, glassy, spreading growth.

Agar plates:- colonies spread concentrically, dry and glassy, and usually firmly attached to the medium.

Gelatin:- slow napiform liquefaction.

Carbohydrates:- dextrose and sucrose are fermented to acid; lactose is unchanged.

Classified as Bacillus subtilis (Sternberg) (Ford) (7).

ORGANISM NO. (8).

Morphology.

Long thick rods with slightly rounded ends. Motile, Gram Positive. Spores appear early; they are usually situated in the centre of the organism. In old cultures the cytoplasm is entirely disintegrated, leaving oval, refractile spores.

Cultural Characters.

Agar slant:- thick, creamy, cretaceous growth, slightly rhizoid at edges.

Agar colonies:- thick and round with regular edges. Young colonies are white and change to a lemon yellow or brown when older.

Gelatin:- saccate liquefaction.

Carbohydrates:- glucose is slightly fermented, lactose and sucrose unchanged.

Classified as Bacillus megatherium (DeBary) (Ford) (7).

TYPE 111. RAPID LACTOSE FERMENTERS.

The characters of this culture are common to those of 23 strains each of which had been studied in detail as defined below:

ORGANISM NO. (9).

MORPHOLOGY..

Twenty-four hour plain agar cultures incubated at 37°C were used to determine the morphology. All strains are Gram negative and distinctly rod shaped, with rounded ends; some are slightly thicker than others, the average size being 1-3 microns.long and 0.3 - 0.7 wide. Stain the organism readily with both aqueous fuchsin and methylene blue. No spore formation could be demonstrated on ordinary media. Motility tests were made in semi-solid media, and by the "hanging drop" method from the water of condensation of 24 hour agar cultures. No true motility could be detected. Capsules could be readily seen in forty-eight hour milk cultures, stained by Hiss' copper sulphate method (10).

Cultural Characters.

The cultures characters were studied from 24 hour old cultures at 37°C unless variations are specially mentioned.

Nutrient Broth:- abundant growth giving a pellicle, clouding and sediment, but no colour or distinct odour.

Agar slant:- growth abundant, echinulate, glistening, and slimy; this latter characteristic being especially evident in older cultures.

Agar plates:- the colonies appear after 12 hours. The growth is heavy and greyish, usually heavier in the centre; the edges are undulated and the consistency granular.

Gelatin plates:- 3 days at 22°C. The cultures grow rapidly,

causing no liquefaction, the colonies are round and glistening and of the same general character as on agar.

Gelatin stab:- 3 days at 22°C, growth filiform and slightly beaded, best at the top and brownish in colour, no liquefaction of gelatin up to 2 months.

Potato:- the growth is luxuriant, echinulate and brownish.

BIOCHEMICAL CHARACTERS.

(a) Litmus Milk.

This was fresh separated milk with sufficient azo-litmin as a 2% solution added to give the required tint and sterilized for three successive days in flowing steam.

The cultures were examined at the end of 24 hours, 2, 5 and 7 days. At the end of 24 hours the cultures gave acid but no coagulation, the degree of acid varying. After 48 hours about half of the strains coagulate the casein, the others effecting practically no change. At 5 days all are coagulated, in most cases the coagulum is bleached at the bottom and broken with gas bubbles and whey is beginning to be extruded at the top or laterally. After a week's incubation the coagulum contracts horizontally to about a third of its former diameter in the majority of the cultures, and is surrounded by clear whey.

(b) Plain Milk.

This medium was made from Klim - a skim milk powder - 100 gms of Klim mixed with 1000 cc. of water and sterilized for 3 successive days in flowing steam in 75 cc amounts in flasks.

After being incubated for 24 hours, there is no apparent change. At 48 hours the milk adhers slightly to the bottom and sides, and seems thicker, There is a soft curd and the whey is beginning to be extruded at the sides after 3 days. At 5 days the curd is cracked and digested to about one half of its former size. After ten days the curd is very much shrunken is of a pale burnt colour and is broken into small pieces which tend to float submerged in clear whey.

Carbohydrates.

(a) Gas Production.

To estimate the amount of gas produced dextrose broth fermentation tubes (Smith's) were used. When the column of gas in the closed area became stationary, usually at the end of 48 hours, the amounts of CO_2 and H were measured. The ratio of CO_2 to H was found to be 1.5 - 1.

Gas was produced in the following carbohydrates:- dextrose, maltose, mannit, xylose, arabinose, dulcite, lactose, salacin, sucrose, raffinose, mosite, dextrin, glycerose, starch, and inulin.

(b) Acid Production.

Acid was produced in the following carbohydrates:- (Brom-thymol-blue was used as indicator).

Dextrose, maltose, mannit, xylose, arabinose, dulcite, lactose, salacin, sucrose, raffinose, inosite, dextrin, glycerose, starch, inulin.

(c) Action on Starch. Production of Diastase.

Allen's method (11) was used - 2% soluble starch solution was added to plain agar, then sterilized and poured into petri dishes. The cultures were streaked on these plates, incubated for 2 days at 37°C and 5 days at room temperature, after which they were flooded with a saturated solution of Iodine in 50% alcohol. A large clear area about the streke indicates diastatic action. All cultures were positive to this reaction.

Methyl Red and Voges Proskauer Reactions. (1) (12).(15).

All cultures gave a negative methyl red reaction, and a positive Voges Proskauer reaction.

Indol Production. (2).

Indol was not produced.

This organism is classified as Bact. aerogenes (Escherich) (8).(9).

TYPE IV. SLOW LACTOSE FERMENTERS.

The characters of this culture are common to those of 11 strains each of which has been studied in detail as defined below.

ORGANISM NO. (10).

These organisms when first isolated from the water required 3 to 4 days to ferment lactose to acid and gas, only a small amount of gas being formed. After half a dozen transfers cultures split the lactose to acid within 48 hours, in

this respect resembling the slow fermenting types of colon described by Bronfenbrenner (13) [illegible] and Sadler & Mounce,(14) In no case was it found possible to materially increase the amount of gas produced either by repeated subcultures or prolonged incubation at 37°C or 22°C.

Morphology.

Twenty-four old agar cultures showed short rods averaging 1-2 microns long by 0.3 -0.7 wide. They were Gram negative and stained readily with the ordinary alcohol and aqueous stains. Motility tests were made on semi solid media, and by the "hanging drop" method from the water of condensation of 24 hour old agar cultures. True motility could not be demonstrated. No capsules were found. All strains were aerobes and facultative anaerobes.

Cultural Characters.

Agar slant:- growth good, spreading, thin, transparent, glassy in old cultures.

Nutrient broth:- heavy viscid sediment, slight clouding, no pelicle.

Agar plates:- the colonies appear early. They are white convex with regular edges, and have a granular consistency.

Gelatin stab and plates:- growth is of the same general character as on the agar. No liquefaction of the gelatin at the end of 2 months.

Potato:- growth good, medium rendered brownish.

BIOCHEMICAL CHARACTERS.

Action on Milk.

Litmus Milk.

This type turns the milk slightly acid after 24 hours growth, but not enough to cause coagulation. No other change is noticed. The cultures were examined for 2 months.

Carbohydrates.

(a) Gas Production.

To estimate the amount of gas produced Smith's fermentation tubes with dextrose broth were used. The column of gas in the closed area was too small to accurately measure the ratio of CO_2 to H. Approximately the amounts of CO_2 and H were equal.

A small amount of gas was produced in the fermentation of dextrose, maltose, mannit, xylose, arabinose, dulcite, lactose, salacin, sucrose, raffinose, inosite, glycerose,

(b) Acid Production.

Acid was produced in the following sugars using Bromthymol-blue as indicator:-

Dextrose, maltose, mannit, xylose, arabinose, dulcite, salacin, sucrose, raffinose, inosite, glycerose.

No acid was produced in starch, inulin or dextrin.

Methyl Red and Voges Proskauer Reactions. (1) (12) (15).

All cultures gave a positive methyl red reaction and a negative Voges Proskauer reaction.

Indol Production.

The Indol test gave a faintly positive reaction. This organism is tentatively classified as a variant of B. neapolitanus (16) differing from it by motility, action on milk and on inesite.

TYPE V. IDENTIFICATION OF NON-LACTOSE FERMENTING BACTERIA.

This group of bacteria gave an alkaline reaction in lactose broth. All are members of the fluorescent group of bacteria, (17).

Morphology.

The vegetative cells twenty-four hours old and grown on plain agar are both long and short rods, often forming short chains. They are uniformly Gram negative and stain equally well with the ordinary aqueous and alcohol stains. Motility was observed in all cultures by the "hanging drop" method, and in semi-solid media this latter being incubated for 24 hours before being inoculated. Capsules were found in about 20% of the cultures, by means of "Hiss" copper sulphate method, (10) milk being used as the medium. No spores could be demonstrated. All strains were aerobes and facultative anaerobes.

Cultural Characters.

All cultures were incubated for twenty-four hours at 37°C, unless variations are mentioned.

Agar slant:- all strains produced a distinct fluorescence; the growth was abundant, glistening, echinulate.

Agar plates:- the growth of the colonies is good, the edge irregular and elevation flat with raised edges. The colour a greyish white at first and later changes to the characteristic fluorescent green. At 22°C and 72 hours old the colonies are distinctly opalescent.

Gelatin plates:- 72 hours old incubated at room temperature. The gelatin liquefiers give abundant growth, forming large saucer shaped colonies, which are filled with a flocculent growth of noticeable fluorescence. These colonies rapidly spread, soon liquefying the whole plate. The non-liquefying strains form colonies similar to those on the agar plates.

Gelatin stab:- observations were made for two monght; 95 per cent of the strains liquefied the gelatin, the rate and form of liquefaction varying with the different strains. The common forms of liquefaction are saccate, stratiform and crateriform. Growth is abundant and fluorescent.

Nutrient broth:- growth abundant, the medium being clouded and having a greenish pellicle and heavy sediment. The broth is distinctly greenish and the odour putrid. In the cultures which form capsules the medium is viscid.

Potato:- growth good and after a few days incubation spreads throughout the medium, changing it either to a dark brown or green colour.

BIOCHEMICAL CHARACTERS.

Carbohydrates. Glucose gives a small amount of acid but no gas-

other sugars not fermented.

Methyl Red and Voges Proskauer Reactions. (1) (12). (15).

The methyl red test is negative. In the Voges Proskauer test all strains give a bright green gluorescence, the intensity of which varies with different strains.

Indol Production. (2).

Inall cases a red fluorescent pigment is secured, which varies from a pale pink to a deep red. It is interesting to note that the production of this red pigment correlates with the production of the green pigment noticed in the Voges Proskauer test. Cultures which give an intense green fluorescence with the phosphate broth and the KOH give a deep red colour in the indol tests. This red pigment is not indol, as it is not soluble in chloroform also the cultures lack that characteristic odour of putrefaction associated with the production of indol.

Action on milk.

The action on milk divides this large group of bacteria into three classes.

(a) Those which have no action on milk.
(b) Those which peptonize without coagulation.
(c) Those which coagulate the milk and then peptonize the medium.

Litmus Milk.

Fresh separated milk with pure azo-litmin as an indicator was used. The milk was inoculated from 24 hour old agar cultures and incubated at 37°C for two weeks.

(a) In about 4% of the cultures the proteolytic enzyme is absent and the milk remains unchanged.

(b) In 85% of the cultures the milk becomes alkaline and peptonization begins at the top, leaving a ring of green whey which extends downward until the whole tube is digested. In a few instances the whey is yellow, finally changing to a distinct orange.

(c) In the remaining 11% of the cultures, the milk remains alkaline or slightly acid, and is coagulated by a rennin-enzyme and then peptonized.

Plain Milk.

This medium was made by mixing 100 gms of Klim with 1000 cc of water, and sterilized in 75 cc amounts in flowing steam. Twenty-four hour old cultures were inoculated into the milk and the flasks incubated at 37°C.

(a) A slight acrid odour is the only noticeable change in the milk. Observations were carried on for two months.

(b) The majority of these fluorescent bacteria belong to this group, digesting the casein without coagulation. In most cases the action is rapid, being completed often within 48 hours. A dark green, transparent layer is formed which extends downward rapidly, leaving a wrinkled pellicle in the surface and a greenish yellow whey; later this changes to a golden yellow in the most cultures; while a few retain their green colouration. All strains have the characteristic putrid odour.

(c) The milk is first coagulated and then digested. The curd is invariably soft; digestion starts usually after 48 hours, beginning at the top and along the sides. When completed a greenish yellow fluorescent whey remains. In the absence of more detailed studies the strains of type are classified as belonging to the fluorescent group of bacteria.

Efforts were made to discover the possible source of the various types of organisms found in the daily counts, especially as concerns the occasional occurrence of slow and rapid lactose fermenting types. Previous data in the hands of the Medical Officer of Health seemed to indicate that the probable source of these organisms was in Seymour and not in Capilano Creeks. With him, an investigation was undertaken of the conditions immediately above the Seymour intakes. A series of samples of soil and surface water on the west bank of the Seymour Creek above the upper intake were taken as high up as Iron Creek. The exact sampling points are approximately indicated on the annexed map.

In taking samples of the soil, sterile wooden tongue depressors were employed to remove the soil to sterile test tubes. Surface water samples were collected in sterile test tubes. Smples were taken also from the intake, from the settling tanks and from between the screens. After collection, the samples were transported as rapidly as possible to the laboratory so that plating was accomplished within two hours of the

taking of the last sample.

Seymour Creek above the upper intake is a comparatively wide and fairly rapid stream. Just above the upper intake there is a clay bank over which surface water trickles. This extends for a distance of approximately a quarter of a mile above the upper intake close to the edge of the stream. It is succeeded by a series of flats, the mountains receding from the stream for some distance. The banks become considerably higher and at one point, known locally as the "Large Slide", there has been a considerable excavation and erosion of the banks, this being two to three hundred feet at its greatest width. Between the large slide and the clay banks a very much smaller slide has occurred. The large slide is within five hundred feet of Iron Creek. In the slide the various strata can be seen. Nearest the surface there is a looseloam occupying a depth of about a foot; beneath this is a layer of sandstone down to a clay layer about six feet below the surface. The clay layer at this point is about eight inches thick. Below the clay there is a layer of sandstone. The sampling points may be described as follows, their approximate position being indicated on the blue print.

(Description of Sampling points).

I. Surface soil from first clay bank, taken about ten feet above the leven of the stream.

II. Surface water which trickles down the clay bank of Sample

I. but fifty feet further up stream.

III. Clay from this bank - see Sample I - taken about six inches below the surface. (deep sample)

IV. Water from a small spring which flows over this bank - see Sample I - entering the stream about one hundred feet north of Sample II.

V. Surface water collected from clay bank mentioned in Sample I.

VI. Surface water which was trickling over a large decayed log.

VII. Water from a spring which flows through a dense underbrush. This sample was taken inland about one hundred yards from creek, between the clay bank and big slide.

VIII. Sand at a small washout.

IX. Sandstone between slides.

X. Clay collected about three feet above the mouth of the largest washout.

XI. Water at the big washout taken near the outlet.

XII. Soil above the clay layer taken from a surface which had been recently exposed by a big washout. This layer is about a hundred feet higher than the surface of the stream.

XIII. Sandstone taken directly beneath the clay layer at this washout.

XIV. Clay from the layer above the sandstone at big washout.

XV. Surface sand on the north bank of Iron Creek where it enters Seymour Creek.

XVI. Water from Iron Creek. This stream is about ten feet wide where it enters Seymour Creek, but fairly shallow. It is called "Iron" Creek because of the peculiar red silt deposited in its bed.

XVII. Subsurface sand on the opposite bank of Iron Creek, taken twelve inches below the surface.

RESULTS OBTAINED.

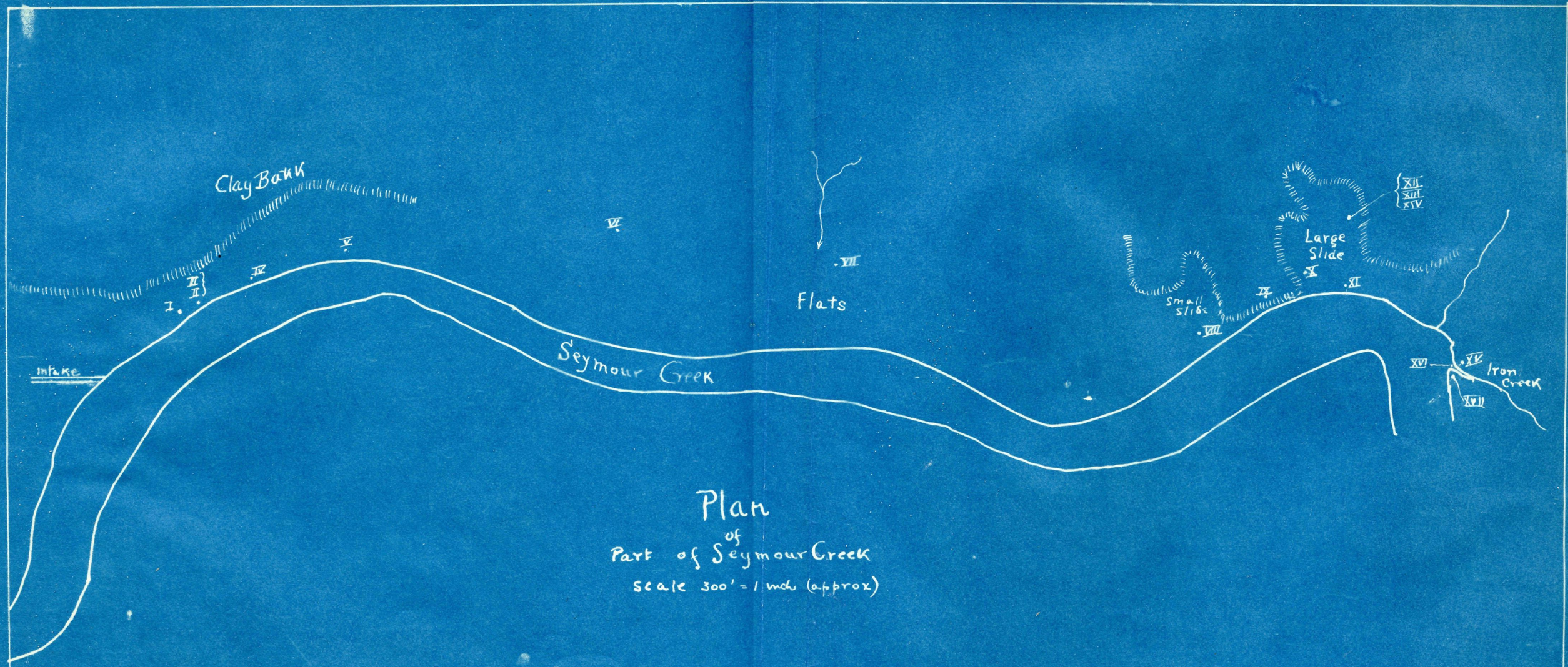
The organisms especially sought for were the lactose fermenters and the fluorescent organisms. No specific attention was paid to the cocci forms. The aerogenes type of organism was found in the following situations - Samples Nos, 1, 2, 3, 4, 5, 7, 8 and 15. The fluorescent type of organism was found in all samples except Nos. 12 and 13. The slow lactose fermenting type was found in Samples 2, 3, 6, 9, 10, 11, 12, 14, and 17. Sample No. 13 showed no growth of any kind. This sample is from the sandstone beneath the clay layer at the "Large Slide", which would indicate that the clay layer was acting as a filter against any organisms in the soil above the clay.

From the studies of these samples it seems that the fluorescent type of organism is found almost constantly in all samples. The aerogenes type of organism is found in samples taken from surface water or surface soil. Where deeper soil samples were taken the slow lactose fermenting type and not the aerogenes type of organism is found; and this slow lactose

fermenting type is found in all samples of water. It is interesting to find that at the intake and in both the east and west tanks of the upper intake all three types of organisms were found, but that the sample between the screens showed only the slow lactose fermenting types and the fluorescent types of organisms.

CONCLUSIONS.

From the data collected in this investigation it would seem that the fluorescent type of organism found is a water form constantly present in the water supply; that the slow lactose fermenting type is a soil organism which obtains fairly easy access to the supply, and that the aerogenes type is probably a grain or grass form which obtains entrance to the supply only after the watershed has been drenched with a considerable rainfall.



Plan
of
Part of Seymour Creek

scale 300' = 1 inch (approx)

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