Surface Taint in Butter

by

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Contents

Introduction.

A problem of great importance to the Dairy Industry is the control of a flavour defect in butter, commonly known to the trade as Surface Taint. This particular type of deterioration has been recognized in Canada since it was first described by Marker(31) in 1919. It first demonstrated itself on the Vancouver Market in a consignment of First Grade pasteurized butter shipped from a reputable Alberta Creamery.

Although the defect under this name was first described in 1919, there is no doubt but that a condition identical with or similar to surface taint had been previously observed and described under other names. As far back as 1899, butter having a "peculiar foetid odour" was described by Gilruth(17) of New Zealand. Eckles (12) in 1900 published a report on butter produced in Iowa possessing "a disagreeable taste with a putrid smell". In 1910, Orla-Jemsen recorded the occurrence in Denmark of a butter with "a peculiar putrid odour". The term "rabbito" used to define a defect reported in Australia is prob ably synonymous with the term surface taint as employed in Canada. Other terms have been used to describe the defects and include "cheesy", "limburger", and "surface flavour". The various appelations that have been employed to describe the defect would appear to depend on the stage of its development in butter. The odour and flavour are characteristically of a putrefactive nature, although rancidity quite often develops subsequent to the appearance of the defect. The term "sweaty feet" most nearly defines the odour of surface taint.

Since the defect was first recognized in Canada, the frequent reports of its occurrence, particularly in butter manufactured in the Prairie Provinces has made the identification of the causative agents and control of the defect an urgent problem. Work on surface taint has been deligently pursued by members of the Division of Dairy Research of the Dominion Department of Agriculture in co-operation with workers from the Provincial Universities and Departments of Agriculture.

As a large importer of butter from the Prairie Provinces, British Columbia has been much concerned with the question of the control of the defect. The occurrence of surface taint in butter manufactured in British Columbia was first reported in 1938, and has served to stimulate work on the problem of the cause and control of the defect within the Province.

PART I - The Bacteriology of Surface Taint.

Historical

Throughout the literature on the putrefactive deterioration of butter it is generally recognized that bacteria play an important role in the development of the defect.

In 1899, Gilruth of New Zealand (17) described the development in butter of a peculiar foetid odour when held at 65° F. for 36 hours. He attributed the defect to the presence of water bacteria, particularly Bacillus fluorescens liquefaciens.

Eckles (12), in 1900, studied an outbreak of putrid butter in Iowa. Several species of organisms, capable of producing a putrid condition in butter when inoculated into cream prior to churning, were isolated. One of the species is described as Bact. fluorescens liquefaciens.

Orla-Jensen (28), in 1910, observed butter possessing a "peculiar foetid odour that ruined it for table use". Bacteria were shown to be responsible for this defect.

In work done by Sadler and Vollum (31) mention is made that in 1919, C. P. Marker, Dairy Commissioner for the Province of Alberta, had found deterioration of butter during storage. They state that: "When boxes of the butter were opened, flavour and odour of a most undesirable nature were evinced, the flavour being defined perhaps most closely as "putrid". The butter was turned out of the boxes, and portions of the outside layer of the respective blocks of butter were removed. Neither flavour nor odour could be detected when the newly cut surfaces were examined. In due course, however, the putrid flavour and undesirable odour developed in the butter which had been newly

exposed".

Sadler and Vollum isolated a number of microorganisms from butter showing this deterioration, and from water and cream at various stages in the manufacture of butter at the creamery troubled with this problem.

A number of their cultures were classified as varieties of the species. B. coli, B. neopolitanus, B. aerogenes, and B. communior, and other cultures, which they did not attempt to identify were described as Gram negative, motile rods, digesting milk, producing a putrid odour, and failing to liquefy gelatin.

Three spore-forming strains studied ceased to exhibit the ability they possessed of producing gas from glucose, after having been held in artificial media for some time. Two of these cultures were erratic in their Gram staining characteristics.

A neutral clot, digestion, and development of a putrid odour were characteristic of these three cultures. They were isolated from deteriorated butter, cream and water supplies.

Spitzer and Parfitt (34), in a study of the action of proteolytic organisms in butter, found that cream inoculated with B. ichthyosmius produced an off flavour and odour when ripened. Unsalted butter made from cream inoculated with B. ichthyosmius produced an unclean, stale, cheesy odour and flavour after 180 days storage. When the butter was salted, a stale, tallowy and bitter flavour developed. Cheesiness failed to develop in the case of salted butter. These workers also reported the presence of undesirable odours and flavours in butter made from cream inoculated with B. proteus vulgaris.

It was found that of the various types studied, the

proteolytic group of organisms were inhibited least by an increased percentage of salt in the butter.

Shutt (33), in 1929, while examining water supplies of creameries producing surface flavour, found large numbers of putrefactive bacteria, chief of which was Pseudomonas fluorescens. When this microorganism was inoculated into sterile butter and incubated at 25° C. for 28 days, surface flavour developed.

Herreid, Macy and Combs (21) reported that a mixed culture which produced cheesiness in butter was found to contain distinct species of Achromobacter, Proteus, Streptococus, and Escherichia, and some resembling Salmonella.

Hammer and Yale (18) in work on the influence of members of the Escherichia - an Aerobacter group of microorganisms on flavour development in butter, found that butter made from cream inoculated with either Aerobacter oxytocum or Aerobacter cloacae developed off flavours when kept at either 7° or 18° C. No off flavours were encountered in the case of Escherichia coli. The defect occurred in both salted and unsalted butter. The character of these off flavours and odours was unclean, resembling somewhat the condition produced in milk by these organisms.

Brown (2) in 1928, studied the spasmodic occurrence of a defect in New South Wales butter. The description of the defect agrees closely with that of surface taint. Bacterial action was shown to be at least partly responsible for the development of the defect.

Hood and White (22), in 1928, in their investigation of surface taint butter reported that a large number of liquefying bacteria which decompose the curd of butter were found in

tainted butter. Surface taint was produced experimentally by inoculation of cream with organisms isolated from creamery water supplies.

Derby and Hammer (10), in 1931, isolated from surface taint butter an organism which was capable of producing the defect experimentally. This organism was tentatively designated as Achromobacter putrefaciens. The fifth edition of Bergey's Manual of Determinative Bacteriology (1937) does not admit the organism to this Genus, but includes it in an appendix under a heterogeneous group of organisms, which, according to Bergey, probably should be placed in some one of the genera included in the family Pseudomonadaceae. Long and Hammer (27) have recently designated this organism as Pseudomonas putrefaciens. *

Organisms other than Achromobacter putrefaciens, which on inoculation into cream produced surface taint in the butter made therefrom, were also isolated by Derby and Hammer. No classification of these organisms was given. Attempts were made to produce Surface taint with Ps. fluorescens. An objectionable condition which may have resembled surface taint appeared for two or three days but rancidity soon developed. These workers were not able to produce the defect by inoculating salted or unsalted butter of high quality with surface taint butter.

Hussong, Long and Hammer (23), in 1937, reported that an organism identified as Ps. fragi is occasionally responsible for cheesiness in unsalted butter.

* Throughout the historical sections, the original genus name is employed to conform with the name in the various publications.

J. Campbell (3), in 1939, isolated a number of organisms from surface taint butter, cream, and water supplies of creameries encountering difficulties with surface taint. He was able to reproduce the defect experimentally with a number of these bacteria, especially with two organisms later classified by L. Campbell, as a strain of Proteus ichthyosmius, and a species of Pseudomonas respectively. Other species isolated were classified as Aerobacter aerogenes, and species of Achromobacter.

Cullity and Griffin (8), in 1938, cited the early work of Loftus-Hills, Scharp and Searle, of Australia, in which rabbito in butter was produced by organisms isolated from factory water supplies, churns, and raw and pasteurized cream. Outbreaks of rabbito investigated by Cullity and Griffin were traced to factory water supplies from which they isolated many types of proteolytic microorganisms. An organism closely resembling Achromobacter putrefaciens was obtained from twowater supplies.

In 1939, Wolochow and Thornton (39), postulated that surface taint in butter is the result, not of bacterial growth in or on the butter, but of the presence in the cream at the time of manufacture "of a product of bacterial growth, which is the precursor of the odoriferous substance recognized as surface taint". They presumed that a chemical change took place in butter on storage, but stated that the factors influencing this change are unknown.

Wolochow was forced to adopt this postulate because he was unable to accept the hypothesis that surface taint arises from the growth of bacteria in or on the butter, for the reason that growth of the causative organism is inhibited by a concentration

of 4% salt. He argues that those moisture droplets containing higher salt concentrations would not support bacterial growth, whilst those of lower concentration would permit development of the organisms with consequent patchy occurrence of the surface taint defect, which, however, is always uniformly distributed throughout a box or churning of butter. The fact that difficulty was experienced in producing the defect by direct inoculation of the butter lent support to his thesis.

In studies on the influence of working on commercial churnings, Wolochow and Thornton (39) found that the defect was confined to unworked samples and offered as an explanation of this finding that surface taint in the unworked butters was produced by bacterial growth in the unincorporated moisture of these butters, and that the finished butters were protected from such growth because of the fine moisture dispersion obtained as a result of working. The basis for this reasoning depends not only upon the salt concentration of the dispersed water droplets inhibiting growth, but as well on the size of the droplets failing to permit bacterial multiplication. Further, they were led to conclude that the agent responsible for surface taint was widespread.

In a later study, Wolochow and Thornton (40) reported identical findings, but placed different interpretation on the results. Failure to isolate Achromobacter putrefaciens, the causative Agent, from many of the alleged surface taint butter samples, led them to question the authenticity of their findings. They state that "Putrefactive bacteria of many types probably grow in the free moisture of the unworked butters",

and that "the odours arising from these butters could easily be mistaken for the S. T. odour", and that "the results had questionable bearing on the surface taint problem".

While Derby and Hammer (10) reported that several types of microorganisms were capable of experimentally producing surface taint, their work indicates that the principle microorganisms responsible for the defect is Achromobacter putrefaciens. They showed clearly that special procedures are required for isolation of this species and suggested that failure to isolate Achromobacter putrefaciens from surface taint butter was in a large measure due to difficulties associated with the technique of isolation. The procedure recommended by Derby and Hammer is enrichment in litmus milk, holding at 5° C. until reduction occurred, followed by plating on beef infusion agar.

Claydon and Hammer (6) suggest other procedures for the isolation of Achromobacter putrefaciens. Pasteurized cream is inoculated with the original defective butter and incubated overnight at 10° C. It is then churned, and the resulting unsalted butter is divided and held at 21° C. and 5° C. until the defect is reproduced. When the butter has become putrid, it is plated and the colonies picked into litmus milk. Another isolation method suggested by these workers is a modification of the Burri Smear Culture Technique. Instead of Agar slants, plates of beef infusion agar plus skim milk fat emulsion are poured and allowed to solidify. The plates are marked into six sectors, and each sector smeared with a tiny portion of butter. These plates are incubated at room temperatures for five days. Colonies are thus easily recognized and picked.

In 1941, Long and Hammer (27) evolved a medium more specific for the isolation of Pseudomonas putrefaciens. This medium is a special Ferric ammonium citrate gelatin agar. On this medium Ps. putrefaciens appears as a brown to brownish red or pink colony. They employed the direct smear technique as well as enrichment in litmus milk prior to plating. Using this procedure, Long and Hammer showed that Ps. putrefaciens is widely distributed, not only in dairy products (milk, cream, and butter) but as well in water, soil and creamery equipment.

In 1940, Wolochow and Thornton (40) described an interesting test for the detection of Achromobacter putrefaciens when grown in sterile skim milk. This organism was found to produce a characteristic odour with properties which have not been duplicated by any other organism studied by them. The odour is described as that of "sweaty feet" and has been shown to possess the property of becoming intensified when the milk culture is spread between the fingers and allowed to dry. Raw milk inoculated with Ach. putrefaciens fails to yield the characteristic sweaty feet odour.

Experimental.

The work reported upon in Part I of this thesis describes in detail, the cultural characteristics of the microorganisms employed throughout the study, and includes a description of a suggested procedure for their detection and isolation.

Four of the cultures were originally isolated by Campbell (3) in studies on surface taint in butter, and were later classified by the author (5) as species of Pseudomonas, Achromobacter, Proteus, and Aerobacter. Three microorganisms were isolated during the course of this study and tentatively classified

within the Genus Pseudomonas. Two cultures, Pseudomonas putrefaciens and Proteus ichthyosmius, originally isolated by Hammer were obtained through the courtesy of Professor Hammer. A detailed description of the cultural and biochemical characteristics of these microorganisms is given below: --

Pseudomonas Putrefaciens.

Morphology

Rods; 0.55 x 2.1 u; singly, in pairs, and short chains; Gram negative; Motile by means of a polar flagellum; (confirmed by author).

Cultural Characteristics

Beef Infusion Agar --- Convex, glistening, smooth, translucent, colorless to grayish colony becoming brown to reddish brown on aging.

Special Gelatin Agar - Colony characteristics similar with exception of colour - brown to reddish brown or pink; putrid odour.

Gelatin Stab --------- Crateriform liquefaction.

Beef Extract Broth --- Turbidity, sediment, and thin pellicle.

Litmus Milk ---------- Rapidly reduced, usually 6-8 hours, by active cultures; proteolysis.

Biochemical Characteristics.

Indole ---------- Not produced

Nitrates ---------- Reduced to nitrites

H_2S ---------- Positive

M. R. ---------- Negative

V. P. ---------- Negative

NH_3 ---------- Positive

Fermenting Power ----- Some strains produce no change in any of the various boiullons, while others produce acid but no gas from maltose, sucrose, arabinose, dextrose, galactose, lactose, and levulose.

Lipolysis ---------- Negative

Phosphotase ---------- Rapidly produced in milk (not confirmed)

Growth Conditions

Facultative; grows at 3° - 30° C.; grows in 4% NaCl; some strains in 6 - 8% NaCl.

Proteus ichthyosmius (Hammer)

Morphology

Rods; 0.6 to 0.8 u x 1.0 to 2.1 u; singly; Gram negative; motile by mean of peritrichous flagella. (Confirmed by author)

Cultural Characteristics.

Agar -------- Small, white colonies, darkening with age.

Broth ------- Turbid with gray sediment.

Gelatin Stab- Liquefaction.

Litmus Milk - Acid; reduced. Cultures have fishy odour.

Biochemical Characteristics.

Indole ------ Produced (Confirmed by author)

Nitrates ---- Reduced to nitrites.

M. R. ------- Positive

V. P. ------- Negative.

Fermenting Ability -- Produces acid and gas from dextrose, levulose, galactose, maltose, sucrose, glycerol, salicin, and mannitol.

Growth Conditions

Facultative; opt. temperature 30 C.

Proteus ichthyosmius (Campbell)

Morphology

Rods; .8 x 1.6 u; square ends; singly and in pairs; Gram negative; Motile by means of peritrichous flagella.

Cultural Characteristics

Standard Agar (Tryptone Glucose Extract Agar)
--- Proteolytic, small white, circular colonies.

Davis Broth --- Slimy pellicle; dense growth with slimy sediment.

Gelatin Stab -- Complete liquifaction.

Litmus Milk --- Firm, acid clot; reduction in 2 days with marked proteolysis.

Biochemical Characteristics

Indole -------- Not produced

Nitrates ------ Reduced to nitrites.

H_2S ---------- Positive

NH_3 ---------- Positive

M.R. ---------- Negative

V.P. ---------- Positive

Fermenting Power -- Acid and gas in sorbite, mannite, glucose, sucrose, and salicin; gas

but only slight acid in glucose and maltose.

Lipolysis ------- Positive

Phosphotase ----- Negative

Growth Conditions

Facultative; grows at 23° - 47° C; opt. 30° C; grows in 6% NaCl.

Aerobacter aerogenes

Morphology

Rods; .6 x .7 u; square ends; singly and in pairs; Gram negative; Motile by means of one or two polar flagella.

Cultural Characteristics

Standard Agar ------ Proteolytic; smooth, white colonies.

Davis Broth -------- Gas produced; dense growth; heavy sediment.

Gelatin Stab ------- No liquefaction

Litmus Milk --------Reduced, hard gassy clot; surface proteolysis.

Biochemical Characteristics.

Indole ---------- Not produced

Nitrates ---------- Reduced to nitrites

H_2S---------- Positive

NH_3 ---------- Positive

M. R. ---------- Negative

V. P. ---------- Positive

Fermenting Ability - Produces acid and gas from sorbite, mannite, glucose, sucrose and lactose.

Lipolysis ---------- Negative.

Growth Conditions.

Facultative; grows from 23° C. - 37° C.; grows in 6% NaCl.

Achromobacter.(Species name not given - unlike any species described in Bergey (1).)

Morphology

Rods; .8 x .9 u; rounded ends; singly and in pairs; Gram negative; motile by means of a single polar flagellum.

Cultural Characteristics

Standard Agar ---- Proteolytic; yellowish colonies.

Davis Broth ------ Dense growth; gummy sediment

Gelatin Stab ----- Complete liquifaction

Litmus Milk ------ Proteolysis

Biochemical Characteristics

Indole ---------- Not Produced

Nitrates ---------- Reduced to nitrites

H_2S ---------- Positive

NH_3 ---------- Positive

M. R. ---------- Negative

V. P. ---------- Negative

Fermenting Ability ---- Produces acid in glucose

Lipolysis ---------- Positive

Growth Conditions.

Falcultative; opt. temperature 23° ; grows in 4% NaCl.

Pseudomonas(Species name not given - unlike any species described in Bergey)

Morphology

Rods; .6 x .8 u; singly, and in pairs, and short chains;

Gram negative; Motile by means of a single polar flagellum.

Cultural Characteristics

Standard Agar ----- Proteolytic, smooth flat colonies with a greenish fluorescence.

Davis Broth ------- Dense growth; gummy sediment; fluorescence at 5° - 30° C.

Gelatin Stab ------ Complete Liquefaction

Litmus Milk ------- Alkaline; reduced; complete proteolysis; fluorescence.

Biochemical Characteristics

Indole ---------- Not produced

Nitrates ---------- Not reduced

H_2S ---------- Negative

NH_3 ---------- Positive

M.R. ---------- Negative

V.P. ---------- Negative

Fermenting Ability - Produces some acid in glucose.

Lipolysis ---------- Positive

Growth Conditions

Facultative; grows at 5° - 47° C.; opt. temperature 23° C.; grows in 6% NaCl.

Cultures B 9 and B 13 -- to be classified as strains of Ps. putrefaciens on the basis of identification procedure proposed by Hammer (27).

Morphology

Rods; gram negative.

Tryptic Casein Digest Agar
(Ferric Amm. citrate and Gelatin added)
---- Pink, mucoid, glistening, convex colony.

Litmus Milk ------- Rapidly reduced.

Nitrates ------- Reduced to nitrites.

Fermenting Ability - Do not ferment glucose or lactose.

Odour produced in Butter -- Surface Taint.

Isolation Technique for Surface Taint producing Bacteria.

The difficulty with which surface taint producing bacteria initiate growth on laboratory media, has been responsible in large measure for the slow progress in determining the factors essential to the development of surface taint in butter. From time to time various changes in procedure for the isolation of the causative microorganisms have been proposed, involving alterations in the type of medium and the method of plating.

In studies on the nature of the medium required for the growth of surface taint producing organisms, the majority of the work reported herein was carried out employing Ps. putrefaciens, the most fastidious of the organisms in so far as initiation of growth is concerned. Repetition of Hammer's work (25) confirmed his finding that the modified Burri Smear Culture Technique was superior to the dilution method of plating.

In preparing the special Iron Gelatin Agar described by Hammer, considerable difficulty was encountered in obtaining a satisfactorily clear medium. It was later found that the source of this difficulty was the peptone employed as the nitrogen source. As the work progressed, various types of nitrogen source were employed, including Peptic Casein Digest Broth after Orla-Jensen (29), and Tryptic Casein Digest Broth after Cole (7). For purposes of isolation and detection of Ps. putrefaciens, the agar containing Tryptic Casein Digest Broth was found superior as a medium to the original agar described by Hammer, in which

proteose peptone served as the source of nitrogen.

On this medium initiation of growth of cultures of Ps. putrefaciens, employing either the direct plating or the modified Burri Smear techniques, was much faster and the growth was markedly more luxuriant. This medium has been employed throughout the greater part of the work reported upon in this thesis as a medium for the isolation of surface taint producing bacteria from butter, creamery water supplies, and other sources.

The Detection of Surface Taint Producing Bacteria in Milk.

The work reported upon by Wolochow and Thornton (40) in which an interesting test for the detection of Ps. putrefaciens involving the recognition of a characteristic odour produced by the culture in pasteurized skim milk, suggested that a study of the odour producing abilities of the various types of micro-organisms used in this investigation and capable of producing surface taint in butter, should be undertaken.

For the purpose of this series of experiments, raw milk of high quality was obtained. It was divided into four equal portions. One portion was dispensed in test tubes in 10cc. quantities, and autoclaved at 245° F. for 10 minutes, another portion tubed in the same manner and autoclaved at this temperature for 25 minutes; a third portion was placed in sterile test tubes and pasteurized at 145° F. for 30 minutes, and the remaining portion was tubed similarly and received no heat treatment. These four milks were then inoculated with the following organisms: -- Ps. putrefaciens (Hammer), Prot. ichthyosmius (Campbell), Aerobacter aerogenes, and species of Pseudomonas and Achromobacter, and incubated for 9 days at 23° C. At the end of this time, the tubes were shaken, three or four

drops taken between thumb and finger, and rubbed until the moisture had evaporated, observing the resulting odour meanwhile. The findings are detailed in Table 1.

Consideration of the results shows that with the majority of the microorganisms, the most undesirable odours occur in the case of those cultures inoculated into pasteurized milk. Some odours did develop in certain of the raw and autoclaved samples, but they were much less intense. Ps. putrefaciens produced the characteristic "sweaty feet" odour, Prot. ichthyosmius (Hammer) a definite fishy smell, and Prot. ichthyosmius (Campbell) a combination of the sweaty feet and fishy odours. The other organisms produced less powerful odours, sweaty feet in the case of the Pseudomonas species and fishy and putrid in the case of the Achromobacter species.

Table 1

Organism	Autoclaved at 245°F. for 10 min.	Autoclaved at 245°F. for 25 min.	Pasteurized at 143°F. for 30 min.	Raw
Ps. putrefaciens	Putrid	Putrid	Sweaty feet!	slightly acid
Prot. ichtyhosmius (Hammer)	Slightly Fishy	Slightly Fishy	Fishy!	Fishy!
Prot. ichthyosmius (Campbell)	---	---	Sweaty feet and Fishy!	Slightly Fishy
Aerobacter aerogenes.	---	---	Slightly acid	---
Pseudomonas	---	Trace of fishiness.	Sweaty feet	Suggestion of Sweaty feet
Achromobacter	---	---	Fishy and putrid	---

PART II - Experimental Butters.

Introduction

The sporadic occurrence of surface taint in the manufacture of butter commercially has made difficult the exact defining of the conditions governing its development. Although it is generally agreed that the occurrence of surface taint in high grade butter made from pasteurized cream is due to the activity of microorganisms, the microbiological deterioration of butter is influenced by other factors entering into the manufacturing process. The acidity of the cream, the extent and method of neutralization, the degree of working and incorporation of moisture, and the concentration of salt are factors which have been shown to be of importance in the production of the defect experimentally.

Historical.

Derby and Hammer (10) showed that surface taint was more readily produced in butter made from cream of low acidity than from cream of high acidity. A high percentage of salt in butter usually prevented the development of the defect. They state that "the prominence of surface taint in recent years is probably due the great changes that have been introduced in the methods of the manufacture of butter". They refer specifically to the improvement in the quality of cream used for butter manufacturing, and to the public demand for butter of low salt concentration.

Hood and White (22), in 1928, found that many samples of surface taint butter come within the same acidity range as that of normal butter. In studies on the effect of neutral-

ization on the development of the defect, these workers found that overneutralization or careless methods of neutralization were not responsible for its occurrence. They concluded from their work on the inhibitory effect of salt that "the question of controlling the undesirable organisms by high salting is impracticable in view of the fact that the market for western butter demands a butter of salt content lower than 2.67%".

Sproule and Hamilton (35), in 1937, in studies on surface taint in Ontario butter suggest that possible reasons for the increased incidence of the defect are:

(1) an increase in the percentage of butter made from high quality cream of low acidity.

(2) the increasing tendency towards the manufacture of butter of low salt content.

Wolochow and Thornton (40) found that the limiting acidity for the growth of Ach. putrefaciens in cream was approximately pH 5.6, roughly corresponding to a titratable acidity of 0.3%. They also found that Ach. putrefaciens produced surface taint in pasteurized cream with a salt content up to 2.5%.

Campbell (3), in 1939, found that overneutralization of the cream appeared to have no effect, either stimulatory or inhibitory, on the development of surface taint. A low temperature of neutralization inhibited the development of the defect. In later work Campbell (4) reported that the optimum titratable acidity of cream for the production of surface taint in butter was .11 - .18% titratable acidity.

Derby and Hammer (10) were able to reproduce surface taint when experimental butters made from cream inoculated with

the specific organisms were incubated at 15.6 ° C. (60°F.) for 2 to 4 days, and at 5°C (41 F.) for 7 to 10 days. Hood and White (22) state that surface taint begins to appear in butter eight to ten days after manufacture; the degree of development of the defect depends somewhat on the temperature of previous storage, and more rapid development takes place around 40 ° to 45°F. Wolochow and Thornton (39) reported the development of surface taint in butter stored as low as - 5°C.

Derby and Hammer (10) in making experimental butter, infected the butter by inoculating the specific organism into the cream prior to churning. They were unable to obtain the defect by inoculating normal butter. Campbell (3) was able to reproduce surface taint experimentally by smearing cultures of organisms responsible for the defect on the surface of normal butter.

Sadler and Vollum (31) in their studies on deterioration of butter, inferred from their results that in order to secure a high grade butter, every effort should be made to secure initial cream of the highest quality; cream in which the bacterial flora present has had but little opportunity to produce acidity or other flavours. Campbell (4) showed that the incidence of surface taint in butter made from cream in which the growth of a certain organism had occurred prior to pasteurization and subsequent infection with a surface taint producing organism than in butter made from pasteurized cream to which this "associated" microorganism had not been added. He found that E. neopolitana, Aer. cloacae, Aer. aerogenes, and E. coli were particularly effective in bringing about this precondition for the development of the defect.

Long and Hammer (24) in studies on the influence of working on the dispersion of moisture and its effect on defects in butter showed that bacteria in butter are largely contained in the water droplets, and that their growth is restricted in large measure to these infected droplets. In underworked butter the droplets are relatively large. In thoroughly worked butter they are small and well separated by fat. A decrease in size of the water droplets results in a lessened supply of nutrients available for bacterial growth. These workers were led to conclude that "the distribution of moisture in butter is a factor tending to influence the growth of bacteria, and changes due to bacterial action should take place more slowly in thoroughly worked than in under worked butter ". Because organisms are more active in underworked than in well worked butter, they suggest adequate working as a means of protecting butter against certain defects, including surface taint.

Long and Hammer (26) in further studies on defects in butter found that reworking of unsalted butter made from pasteurized cream inoculated with various organisms, and held at 10°C., usually resulted in an increased bacterial growth, and a decrease in the time required for the development of defects.

They also found that the printing of butter in a type of equipment which subjected it to a reworking process tended to aggregate the moisture droplets. The contamination of previously uninfected water droplets and the provision of a greater food supply by aggregation of the moisture droplets were shown to be important factors in the acceleration of various microbiological changes that take place in butter on reworking. Long and Hammer

further found that reworking of butter after several days storage commonly resulted in a more rapid and extensive increase in the number of bacteria, than in butter reworked shortly after manufacture. Defects were also found to develop faster in butter that had been held longer prior to reworking. Achromobacter putrefaciens was used as one of the organisms in these studies.

Claydon and Hammer (6) were able to develop surface taint experimentally with Ach. putrefaciens in one day at 21°C., and in seven days or less at 5°C. They found that salt, while tending to inhibit this defect was not completely effective unless the butter had been thoroughly worked.

Experimental.

The studies reported upon herein were undertaken with the object of determining the conditions governing the development of surface taint in butter. Consideration was given to the following factors:

1. Type of microorganism.
2. Associative bacterial action.
3. Mode of infection.
4. Acidity of cream.
5. Neutralization.
6. Degree of working.
7. Salt percentage.
8. Reworking.
9. Storage conditions, including temperature and aeration.

PART II A - Experimental Churnings.

Procedure and Methods.

For the experimental churnings, 6-7 pounds of raw sweet cream were standarized to 33% butterfat, pasteurized at 175°F. (79 C.) for ten minutes, cooled immediately and held overnight at 4°C. Just prior to churning, acidity determinations were made.

The cream was churned in a No. 5 Buttercup stainless steel hand churn, sterilized by autoclaving. A churning temperature of approximately 44° - 46° F. was employed. A control sample was taken directly from the churn after the buttermilk had been drained off prior to washing. The butter was then washed twice with sterile wash water, which had been inoculated with approximately .033% of a young Davis (9) Broth culture of the microorganism understudy. Salt was added at the rate of $\frac{1}{2}$ - $\frac{5}{4}$ oz. per pound of butter. The butter was worked on a small wooden hand worker sterilized by chemical treatment.

Butter samples were taken aseptically in sterile 5" glass bottles equipped with air-tight aluminum screw caps.

The organisms employed in this part of the work were representative cultures isolated from surface taint butter, cream, or creamery water supplies by Campbell (3). They included a strain of Proteus ichthyosmius, Aerobacter aerogenes, a species of Pseudomonas, and a species of Achromobacter. Pseudomonas putrefaciens, obtained from Professor Hammer was also employed.

The bacteriological examination of the butters was made by the plate method, using Standard Agar (Tryptone Glucose

Extract Agar). Samples were plated the day of manufacture, and after one week's storage.

The pH of the finished butter serum, obtained by melting the butter, was determined with a quinhydrone potentiometer.

The butter samples were graded at suitable intervals by Mr. H. A. Mason, Dominion Dairy Produces Grader for the Province of British Columbia.

Effect of Degree of Working and Temperature of Storage on Development of the Defect.

Long and Hammer maintain that the incorporation and dispersion of moisture are important factors influencing the bacterial deterioration of butter, and have a direct bearing on the development of defects.

In order to determine the direct influence of this factor on the development of surface taint, samples were therefore taken at the following stages of working.

I. Very slightly worked.

II. Worked slightly more.

III. Slightly underworked.

IV. Sufficiently worked.

V. Extremely overworked.

In order to study the effect of temperature on the activity of the microorganism in butter worked to varying degrees, duplicate samples of each stage of working were taken. One sample was stored under conditions very favourable for the growth of the microorganism - 23°C. (73.4°F.), while the other sample was held at approximately 5°C. (41°F.) for one week and then transferred to 23°C. prior to examination.

Discussion.

The findings are recorded graphically in Figures 1 to 21 in which the logarithms of bacterial counts are plotted against degrees of working.

Although the peculiar odour and flavour characteristic of surface taint was rarely encountered, other equally undesirable odours and flavours were consistently obtained. These were variously described as "Nearly S.T.", "putrid", "dirty", "cheesy", "amine", and "synthetic". The word "synthetic" was used to describe the particular odour which suggested the smell produced by various combinations of synthetic amines in butter, as reported by Campbell (3).

There is no sharp dividing line between these closely related types of odours, since, like other bacterial defects, each undergoes a sequence of changes dependent upon microbiological activity. One defect may predominate and be replaced by another, or a blending may result in a defect suggestive of more than one type.

These undesirable defects occurred more frequently in underworked butter than in well worked butter. When the defect appeared in all or in the majority of the butters at the various stages of working, there was usually a decrease in potency of the odour with an increase in the degree of working. Figs. 3,5,6,9, 11,12,14,15.

Because the incidence of characteristic surface taint is low in this series of experiments, nothing definite can be said with regard to the influence of temperature of storage on its development. However, the production of closely related un-

desirable odours occurred more often in the butter samples that were stored one week at 4°C., and then held at 23°C. for a short period of time, than in those that were kept at 23°C. throughout the holding period. Fig. 1, 2, 11, 12, 13, 14, 15.

Undesirable odours were more often encountered in experimental butters inoculated with cultures of Prot. ichthyosmius (Campbell) and Pseudomonas (Campbell) than when cultures of Aerobacter aerogenes and Achromobacter (Campbell) were employed. Fig. 1 - 4.

Figs. 1 - 21 show clearly the influence of the degree of working and the temperature of storage on the growth of microorganisms in butter. In the case of the bacteriological examinations made on the day of manufacture, a tendency towards a definite decrease in bacterial count as the extent of working increases is to be seen, Fig. 1 - 21. The bacterial counts on the samples held at 5°C. for one week show that for the lesser degrees of working, there has been considerable microbial multiplication in the case of certain of the experimental churnings. The majority of the samples showed little evidence of change, whilst in others the bacterial count was actually lower after one week's storage than on the day of manufacture. Many of these samples were conspicuously tainted. As the degree of working increased, the bacterial counts of the samples stored at 5°C, showed in general a marked decline approximating the count obtained on the original "extremely overworked" butter, Figs. 1, 3, 4, 6, 0 20.

When the butters were stored at 23°C., a marked increase in count is to be seen in the case of practically all

samples, Figs. 1 - 9, 10 - 18, 20. In the samples submitted to "slight working", the relative increase in numbers is higher than in the samples worked to a greater extent. In most cases, even when held at 23°C., the "extremely overworked" samples showed but a slight increase in bacterial count.

Associative Action of Microorganisms.

In work on the influence of the associative action of other organisms on the subsequent activity of surface taint bacteria, the buttermaking procedure was altered to the extent that the raw sweet cream was inoculated with the "associated" bacterium, incubated overnight, and neutralized prior to pasteurization. o.025% inoculum of a young Davis Broth culture of the "associated" organism was employed. Sodium carbonate or bicarbonate was used as the neutralizing agent. The butter was made after the manner described above, employing the wash water as the channel of infection for the surface taint organism. As the work progressed, a further change in technique was adopted. At the fourteenth churning, the amount of salt used was reduced to one third of the amount previously employed. This departure from the previous procedure was adopted as a result of the failure to consistently reproduce the characteristic surface taint defect, and in the light of results reported by other workers (10), (22), (35).

Discussion.

In churnings 5 - 20, the influence of the growth of associated microorganisms with the resultant changes in the acidity of the cream and the neutralization problem thus created along with other pertinent factors to which reference has already been made, have been studied.

Although the characteristic surface taint defect was not encountered in the experimental churnings, a higher incidence of undesirable odours and flavours was found in the case of these butters than in the series in which only surface taint producing organisms were employed.

Subsequent to the reduction in the salt content of the experimental butters, defects closely akin to surface taint were more commonly encountered.

KEY

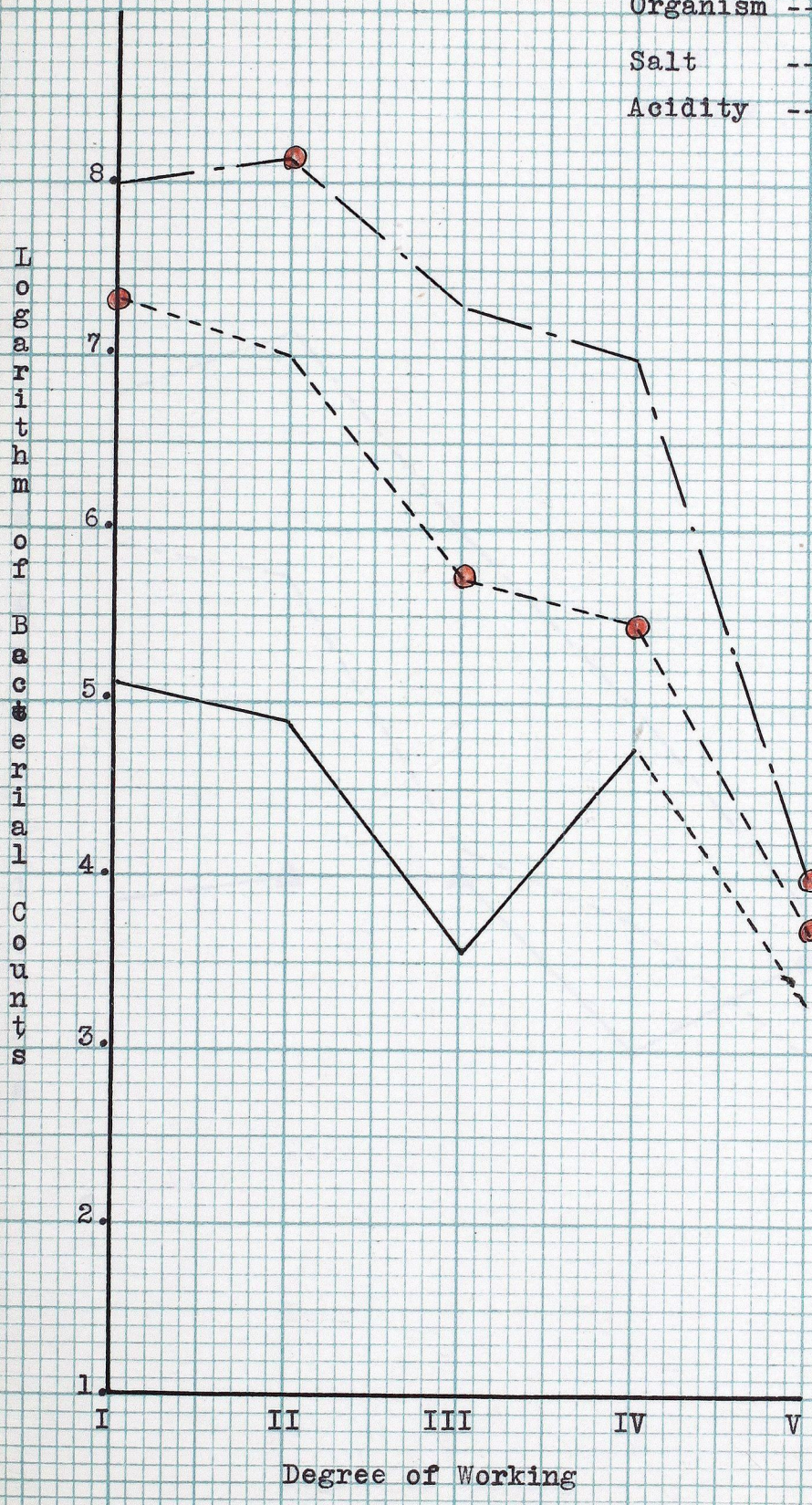
- ------- Surface Taint
- ------- Nearly Surface Taint
- ------- Putrid
- ------- Dirty
- ------- Synthetic
- ------- Amine

In In Figures 14 - 21, the decreased salt concentration is indicated by ======, the original salt concentration by ======.

Fig. 1.

Churning 1.

Organism --- Prot.ichthyosmius (Campbell)
Salt --- 1/8 oz./lb.
Acidity --- .12%



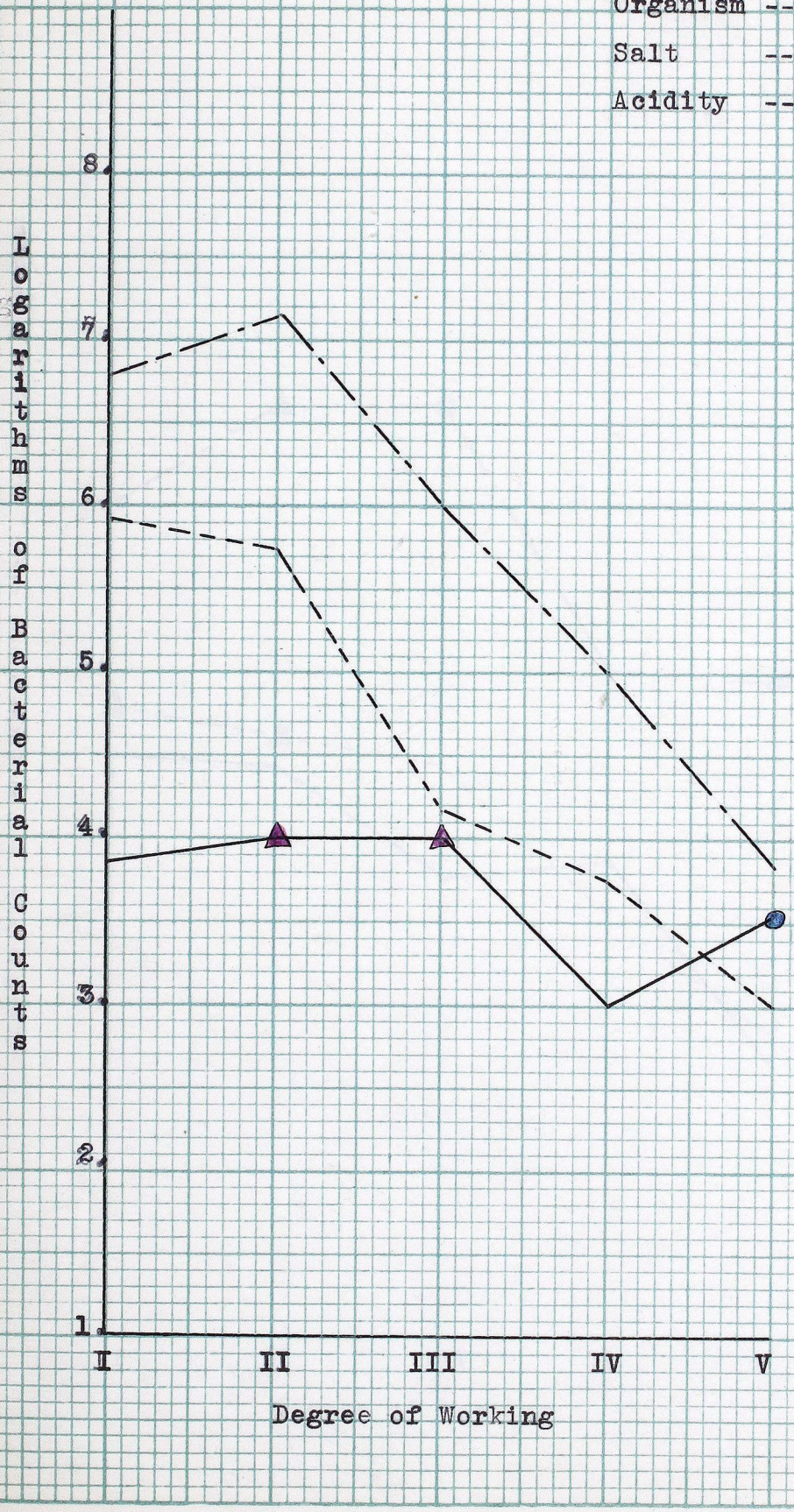
This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.
T.

Fig. 2.

Churning 2.

Organism --- Aer.aerogenes (Campbell)
Salt --- 1/2 oz./lb.
Acidity --- .14%

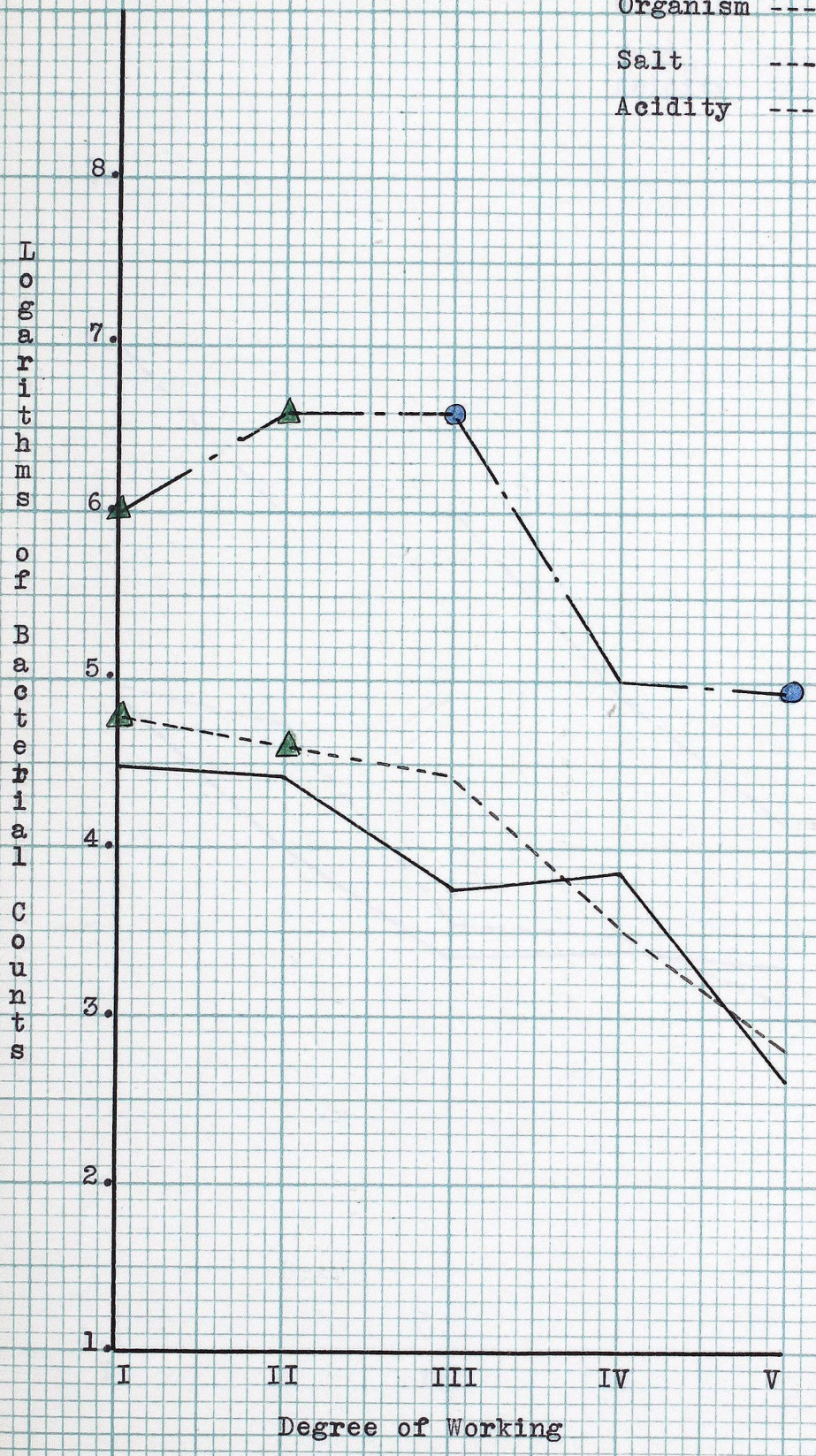


This plate 10 x 7 inches. Edge of each small square 1/10 inch.

Fig. 3.

Churning 3.

Organism --- Pseudomonas (Campbell)
Salt --- 1/2 oz./lb.
Acidity --- .125%



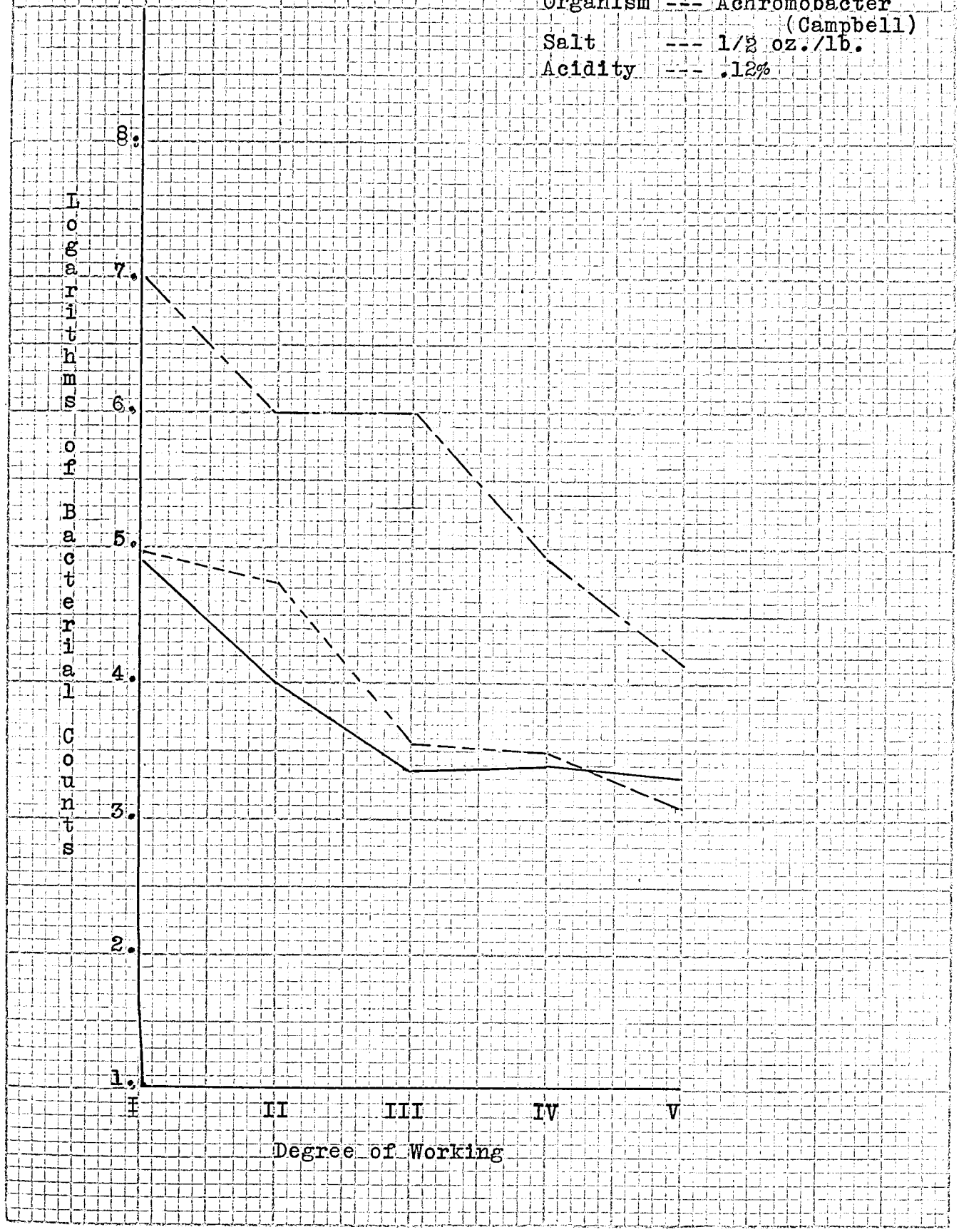
This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.
T.

Fig. 4.

Churning 4.

Organism --- Achromobacter (Campbell)
Salt --- 1/2 oz./lb.
Acidity --- .12%



This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.
T.

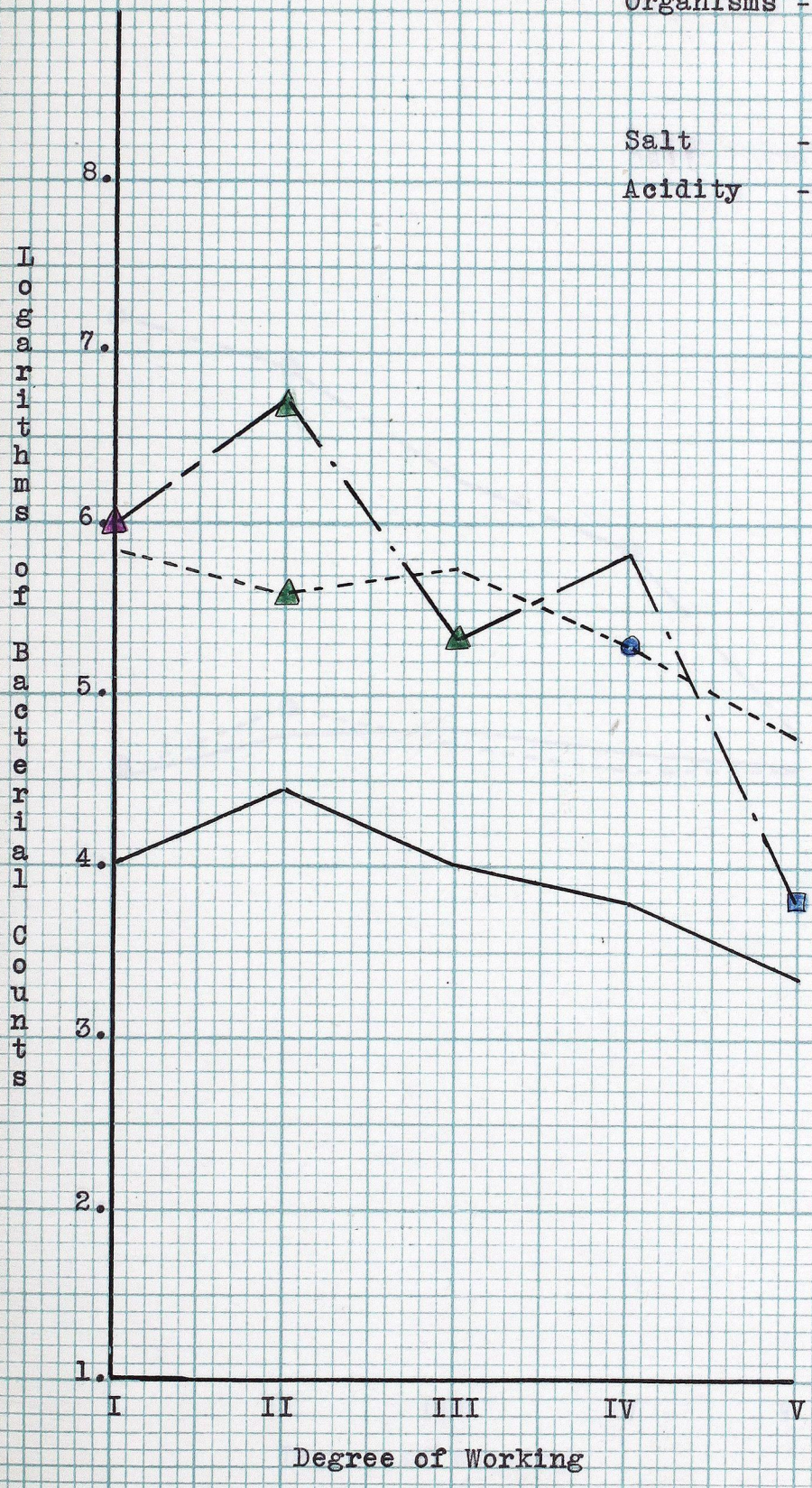
Fig. 5.

Churning 5.

Organisms ---Prot.ichthyosmius (Campbell) and Prot,ichthyosmius (Campbell)

Salt ---1/2 oz./lb.

Acidity ---.125%



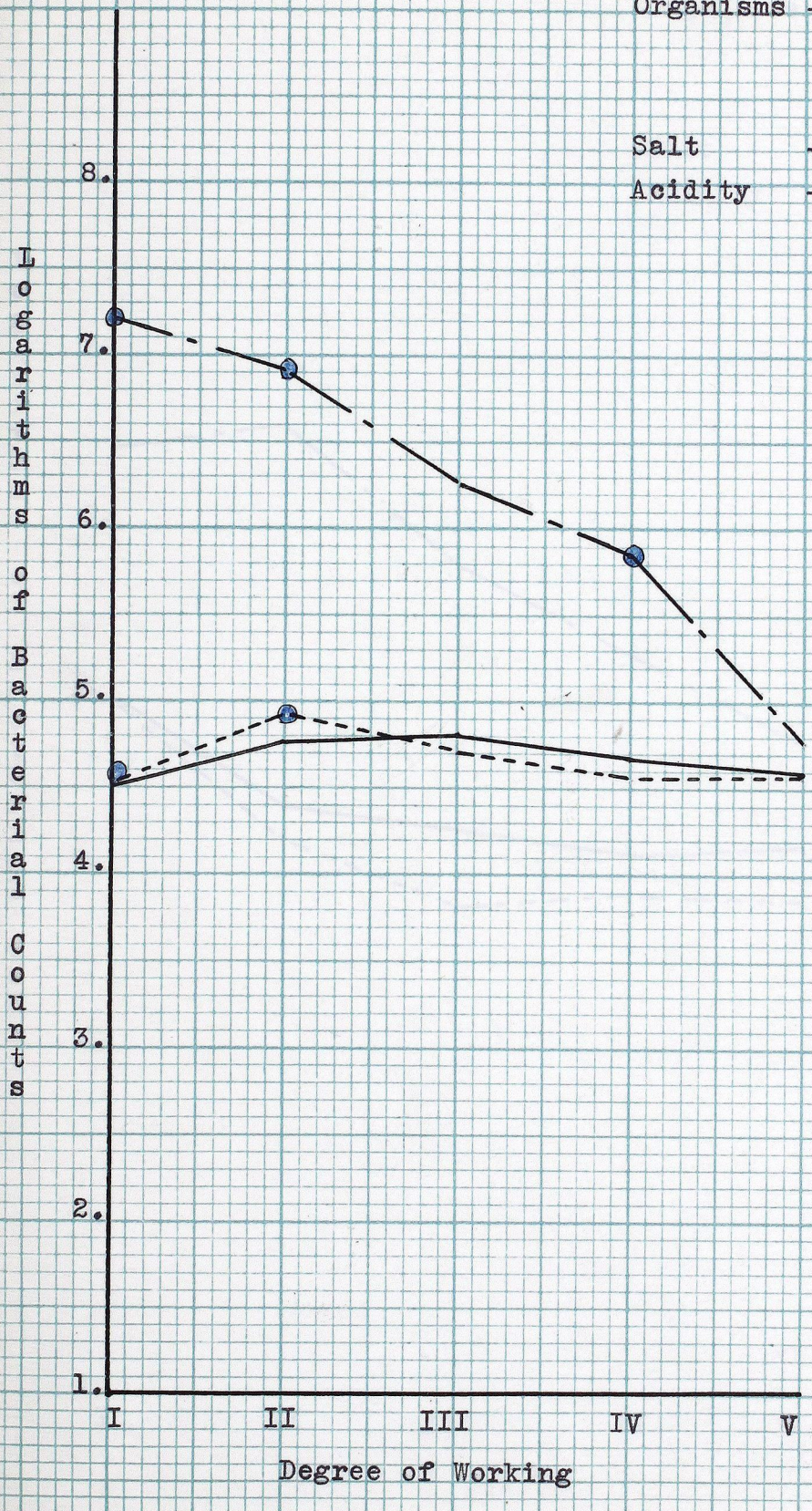
This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.
T.

Fig. 6.

Churning 6.

Organisms --- Aer.aerogenes (Campbell) and Aer.aerogenes (Campbell)
Salt --- 1/2 oz./lb.
Acidity --- .11%

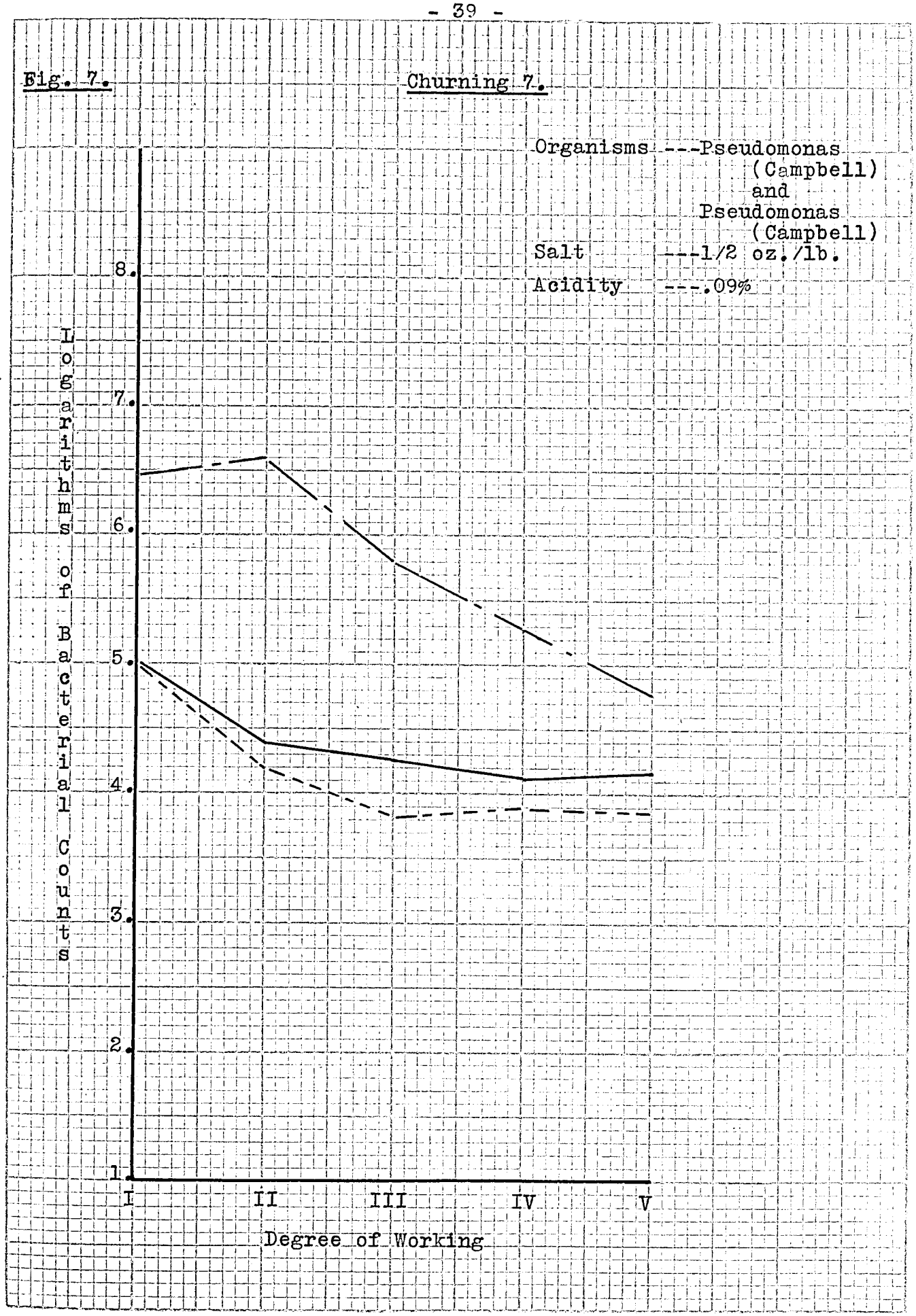


This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.
T.

Fig. 7.

Churning 7.

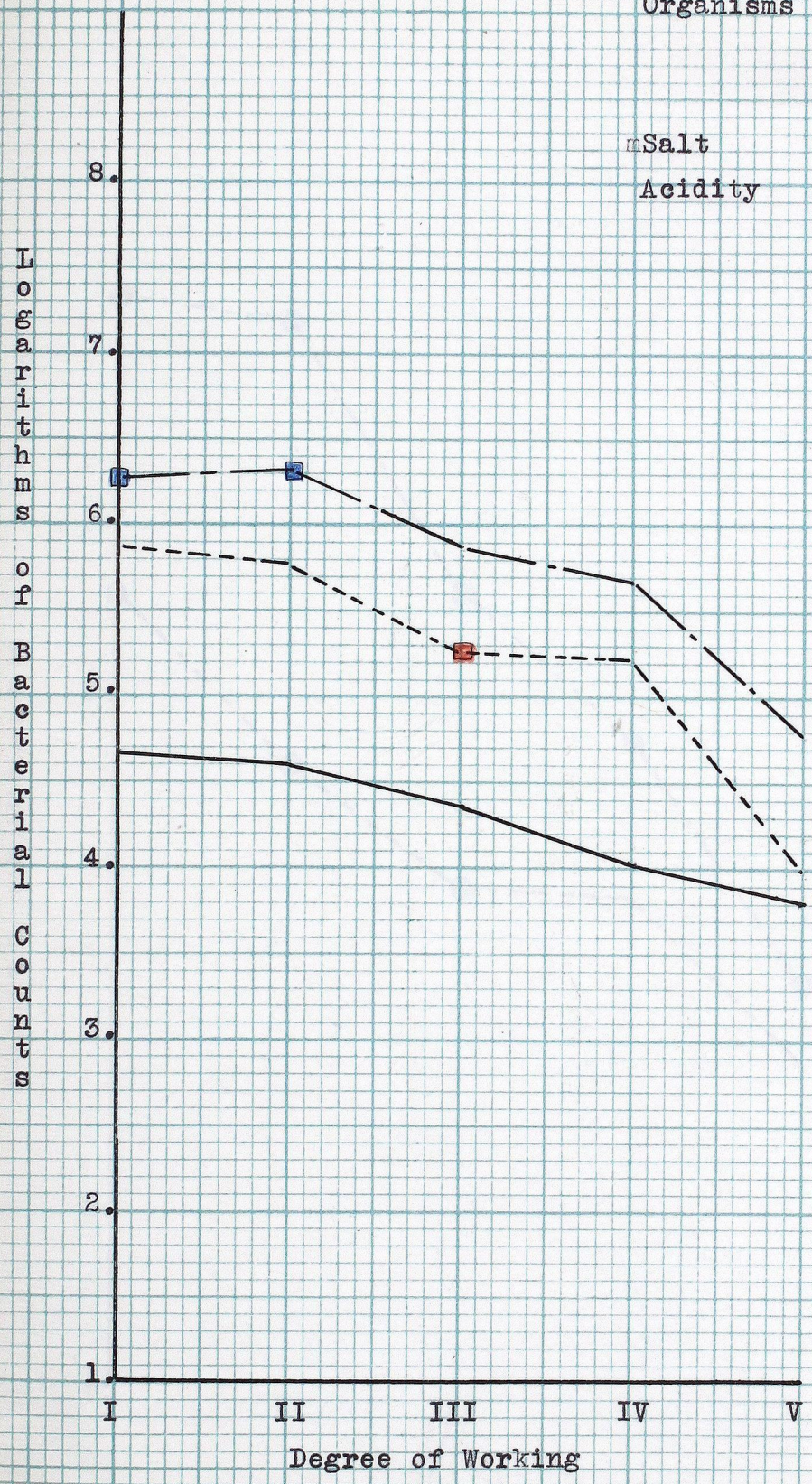


This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.

Fig. 8.

Churning 8.



This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.
I.

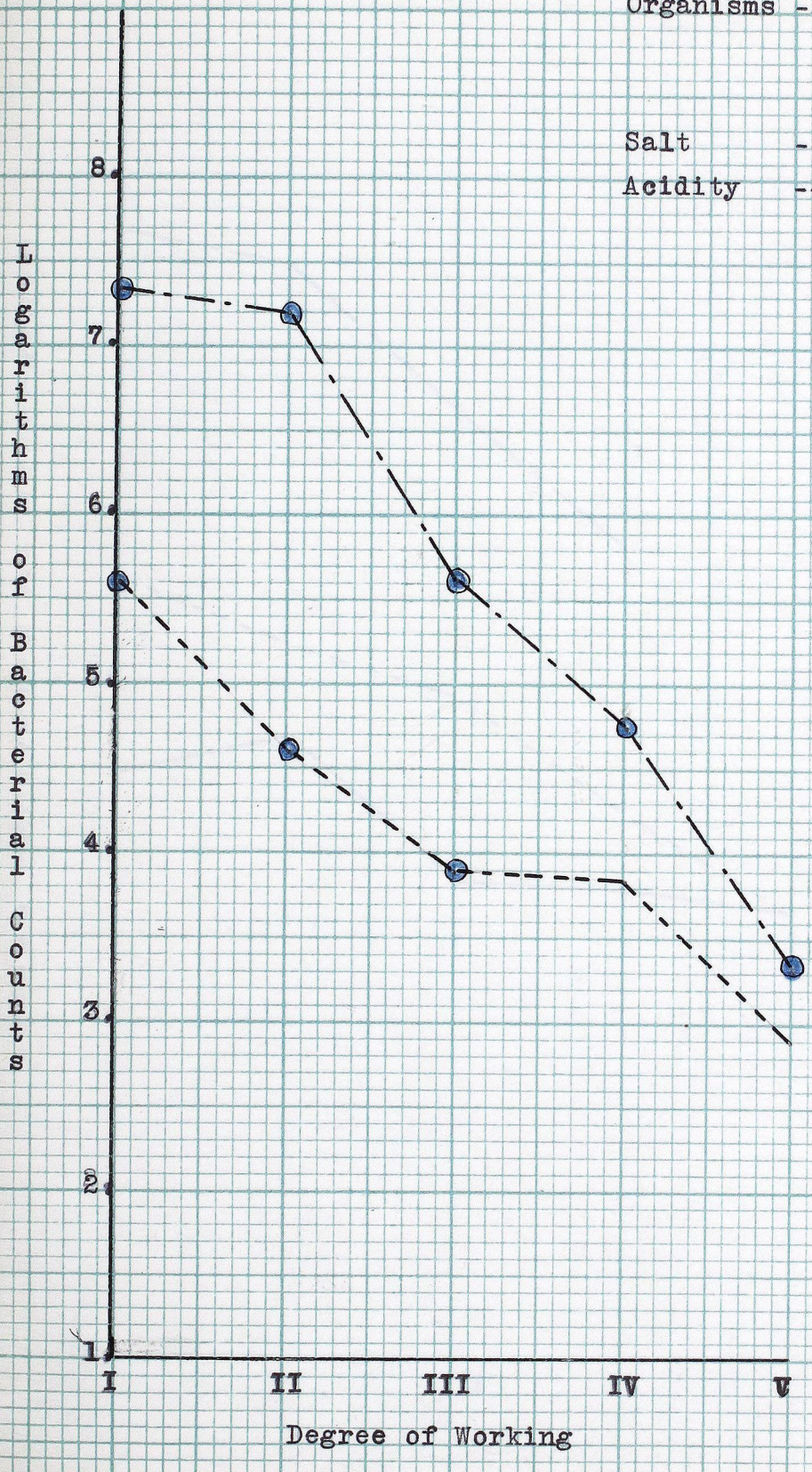
Fig. 9.

Churning 9.

Organisms --- Prot.ichthyosmius (Campbell) and Pseudomonas (Campbell)

Salt --- 1/2 oz./lb.

Acidity --- .10%



This plate 10 x 7 inches. Edge of each small square 1/10 inch.

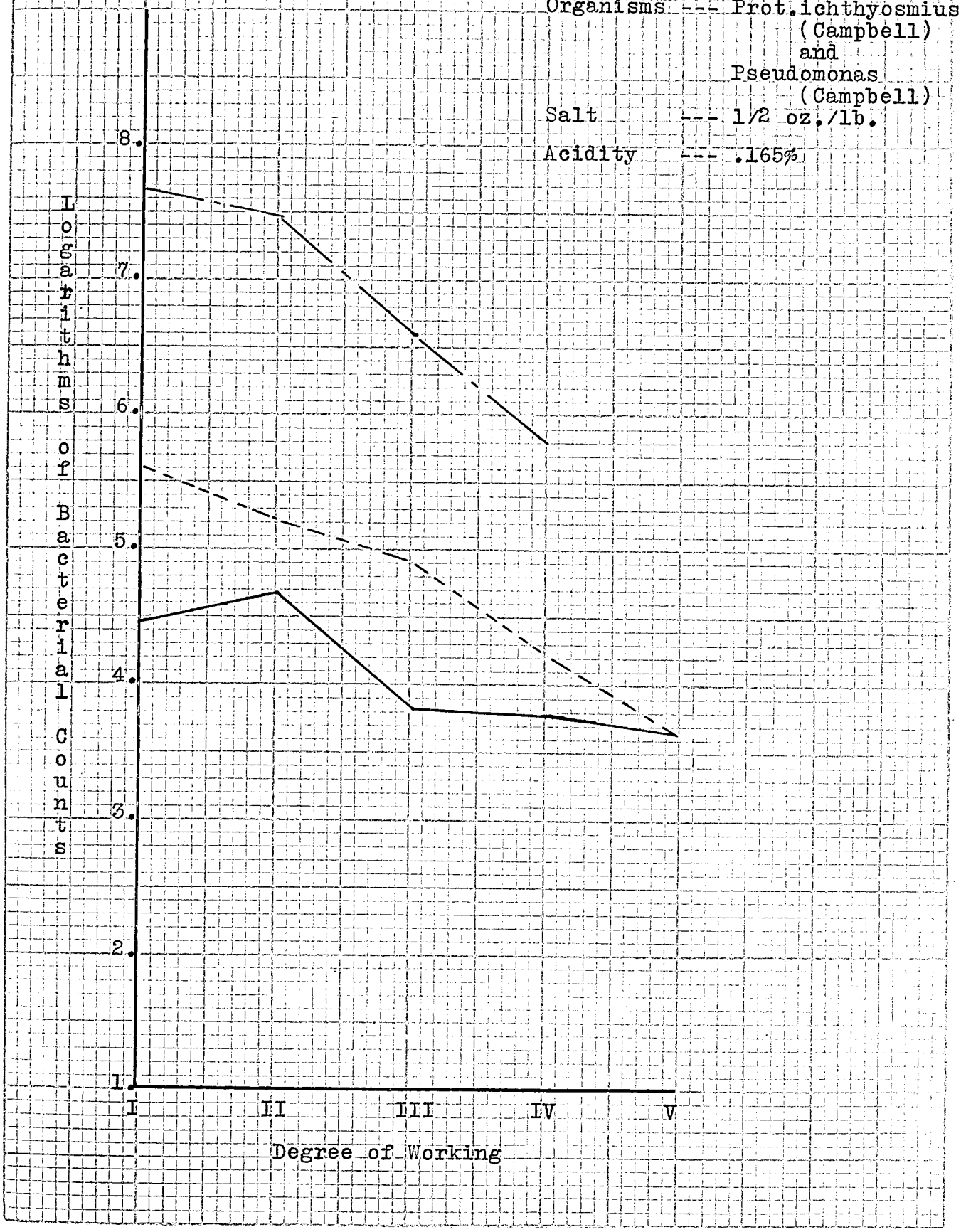
Fig. 10.

Churning 10.

Organisms --- Prot.ichthyosmius (Campbell) and Pseudomonas (Campbell)

Salt --- 1/2 oz./lb.

Acidity --- .165%



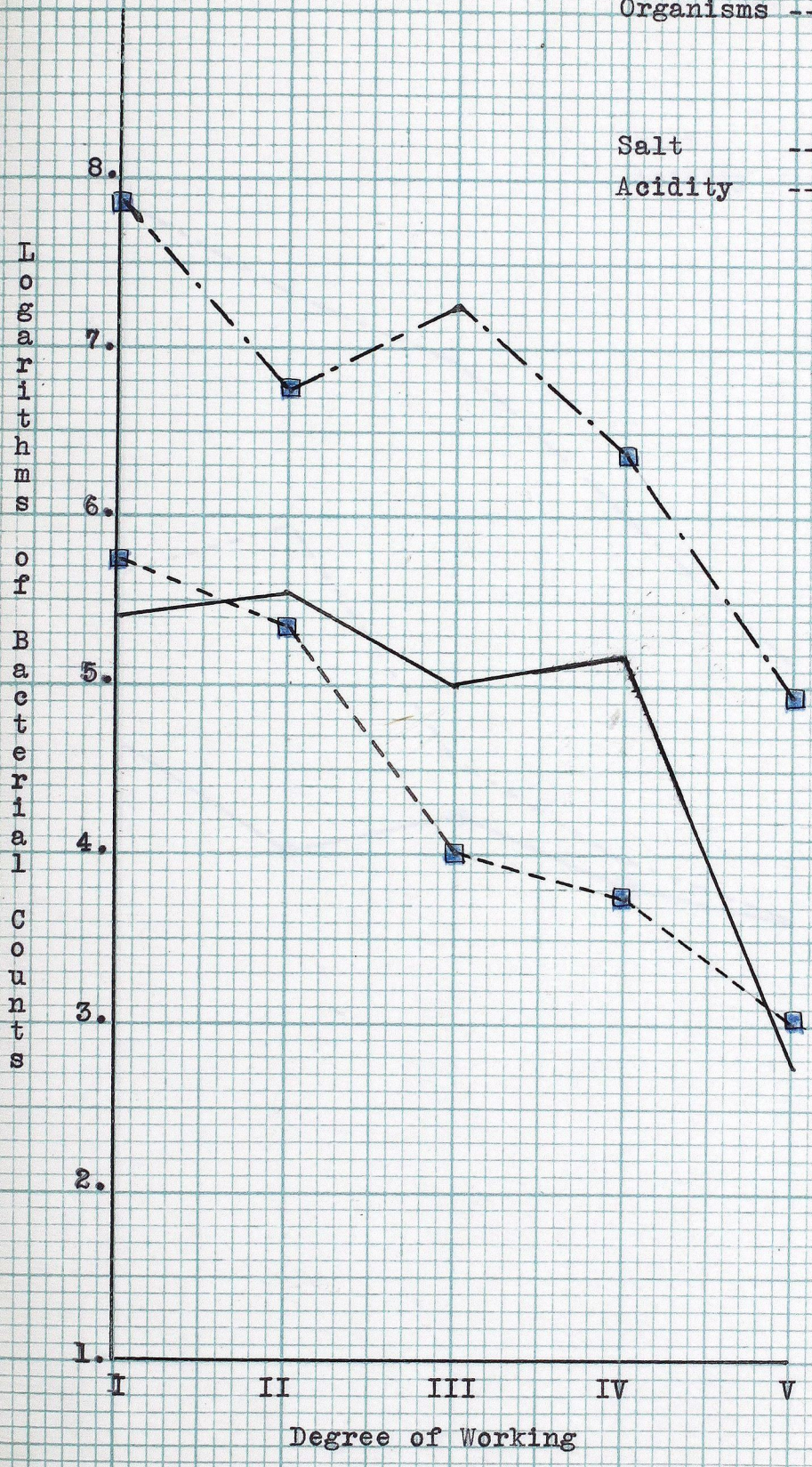
This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.

Fig. 11.

Churning 11.

Organisms --- Prot.ichthyosmius (Campbell) and Prot.ichthyosmius (Campbell)
Salt --- 1/2 oz. /lb.
Acidity --- .09%



This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.

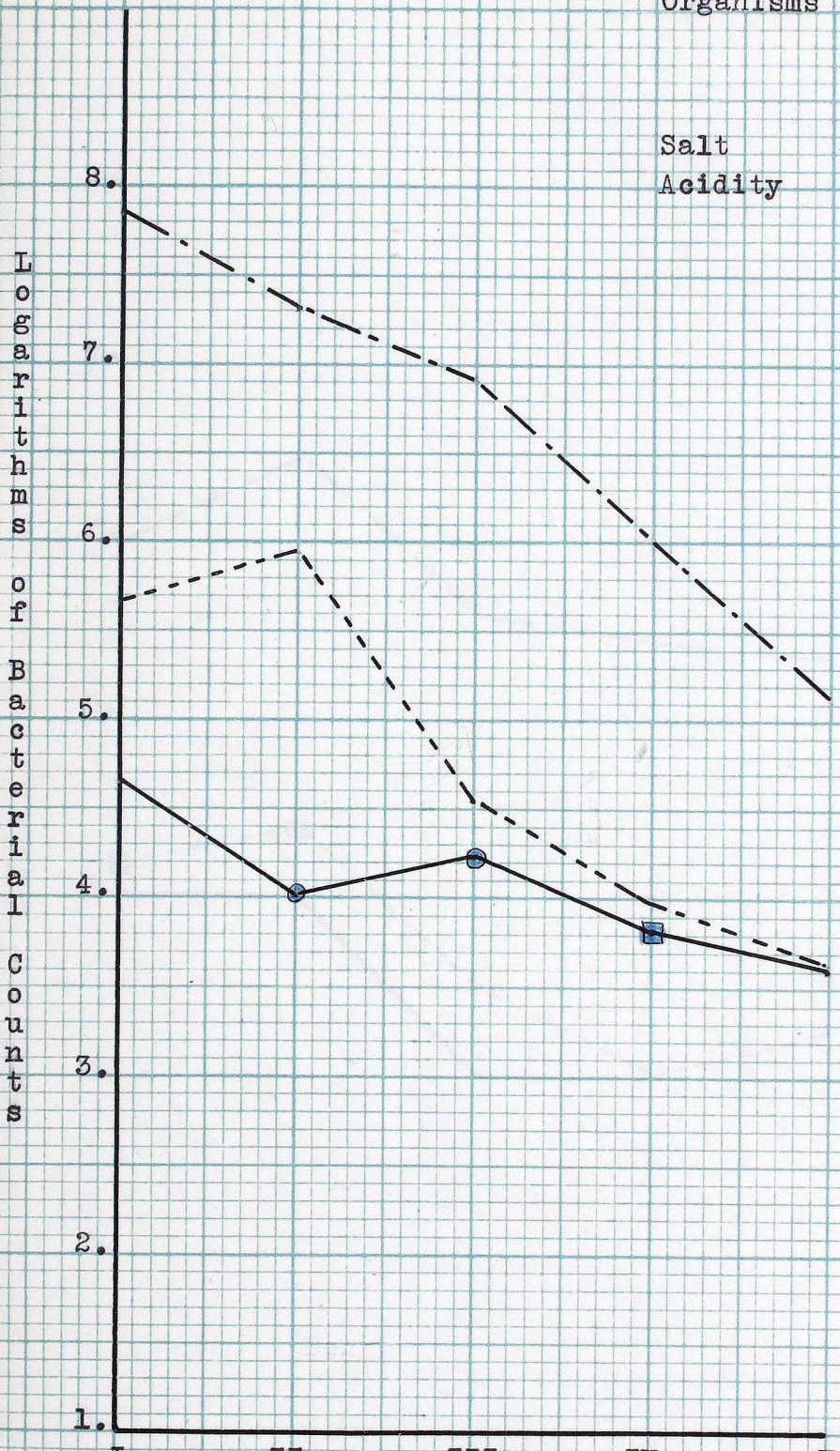
Fig. 12.

Churning 12.

Organisms --- Prot.ichthyosmius (Campbell) amd Aer.aerogenes (Campbell)

Salt --- 1/2 oz./lb.

Acidity --- .115%



This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.
T.

Fig. 13.

Churning 13.

Organisms --- Prot.ichthyosmius (Campbell) and Achromobacter (Campbell)
Salt --- 1/2 oz./lb.
Acidity --- .23%

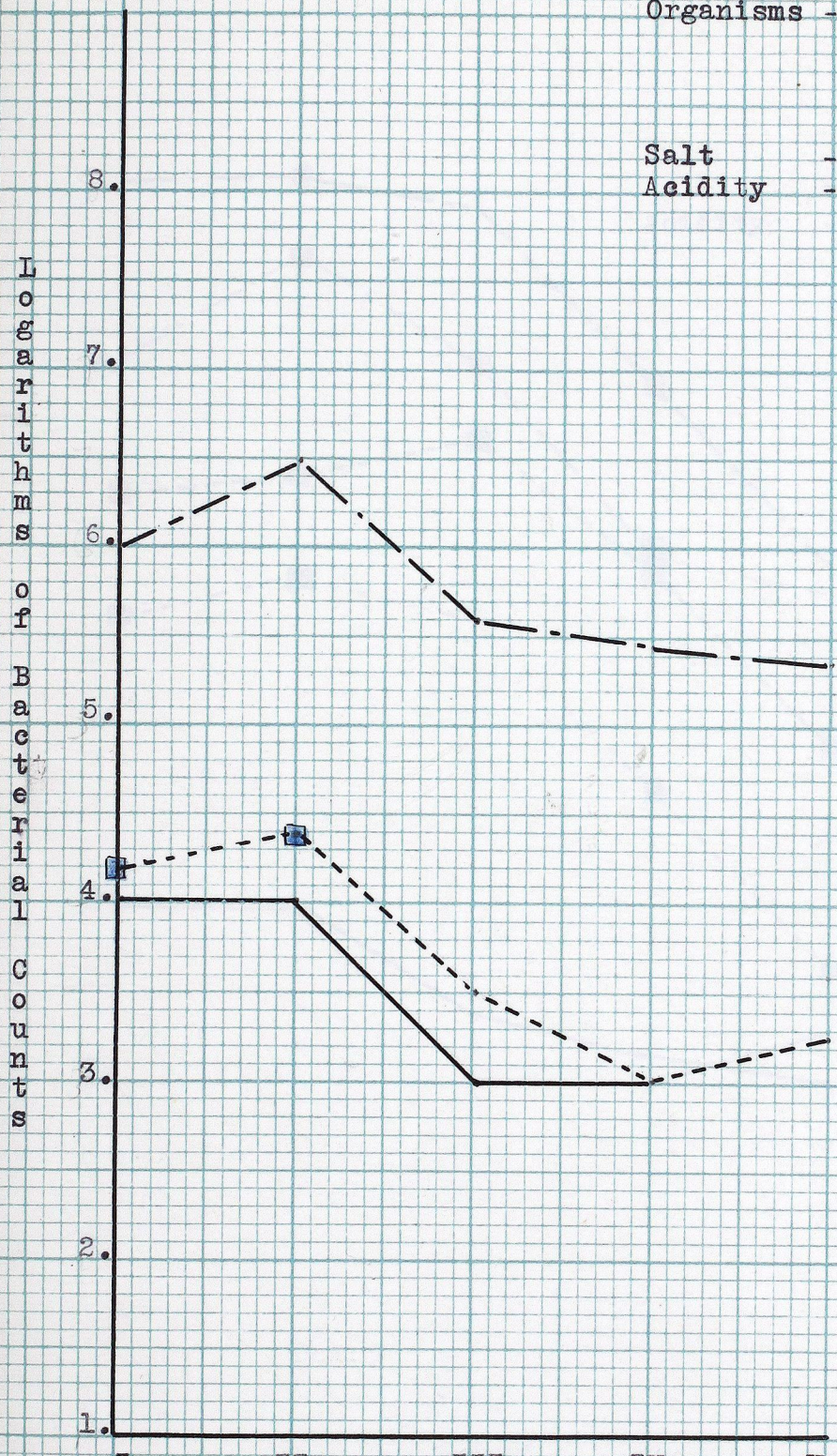
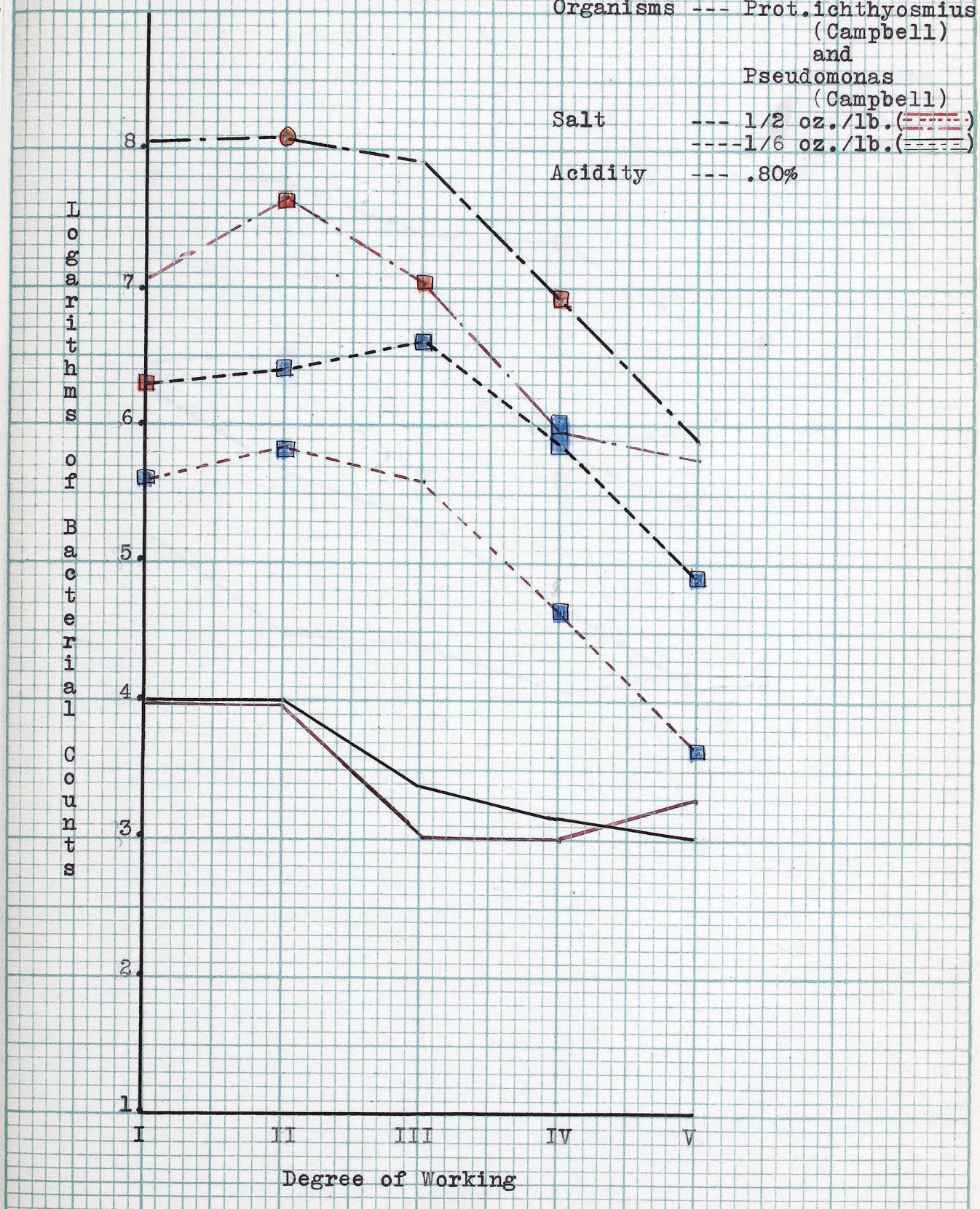


Fig. 14.

Churning 14.

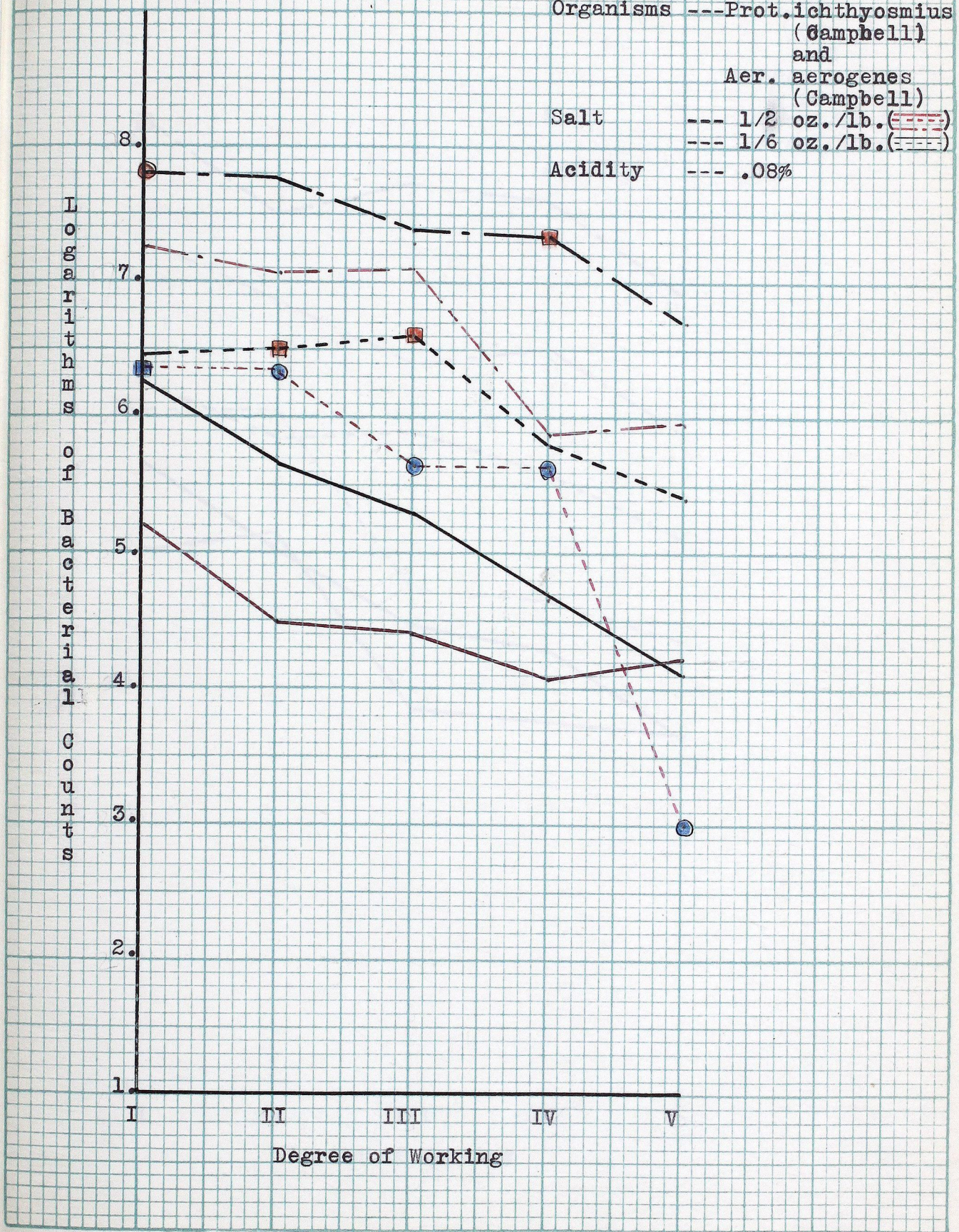


This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.
T.

Fig. 15.

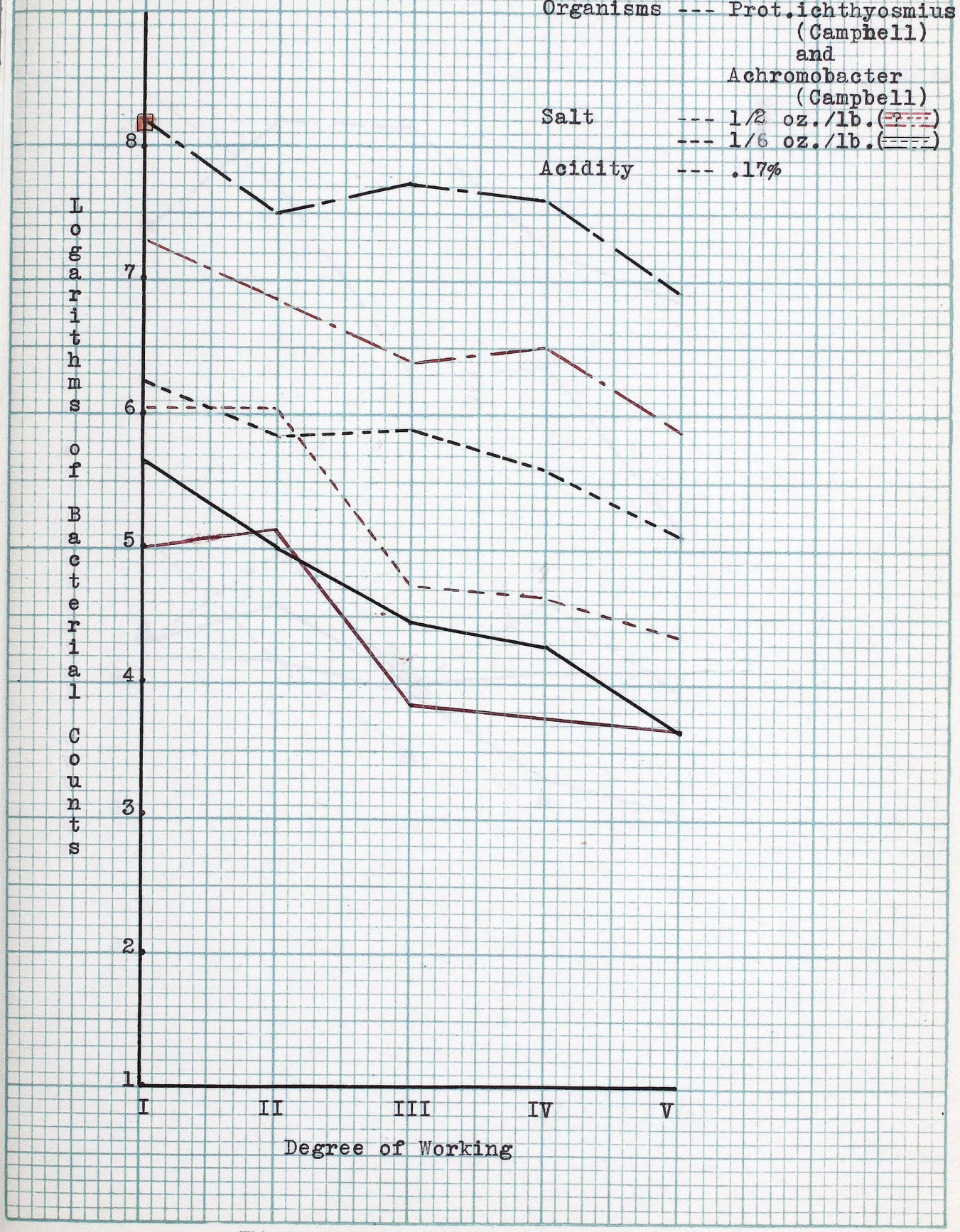
Churning 15.



This plate 10 x 7 inches. Edge of each small square 1/10 inch.

Fig. 16.

Churning 16.



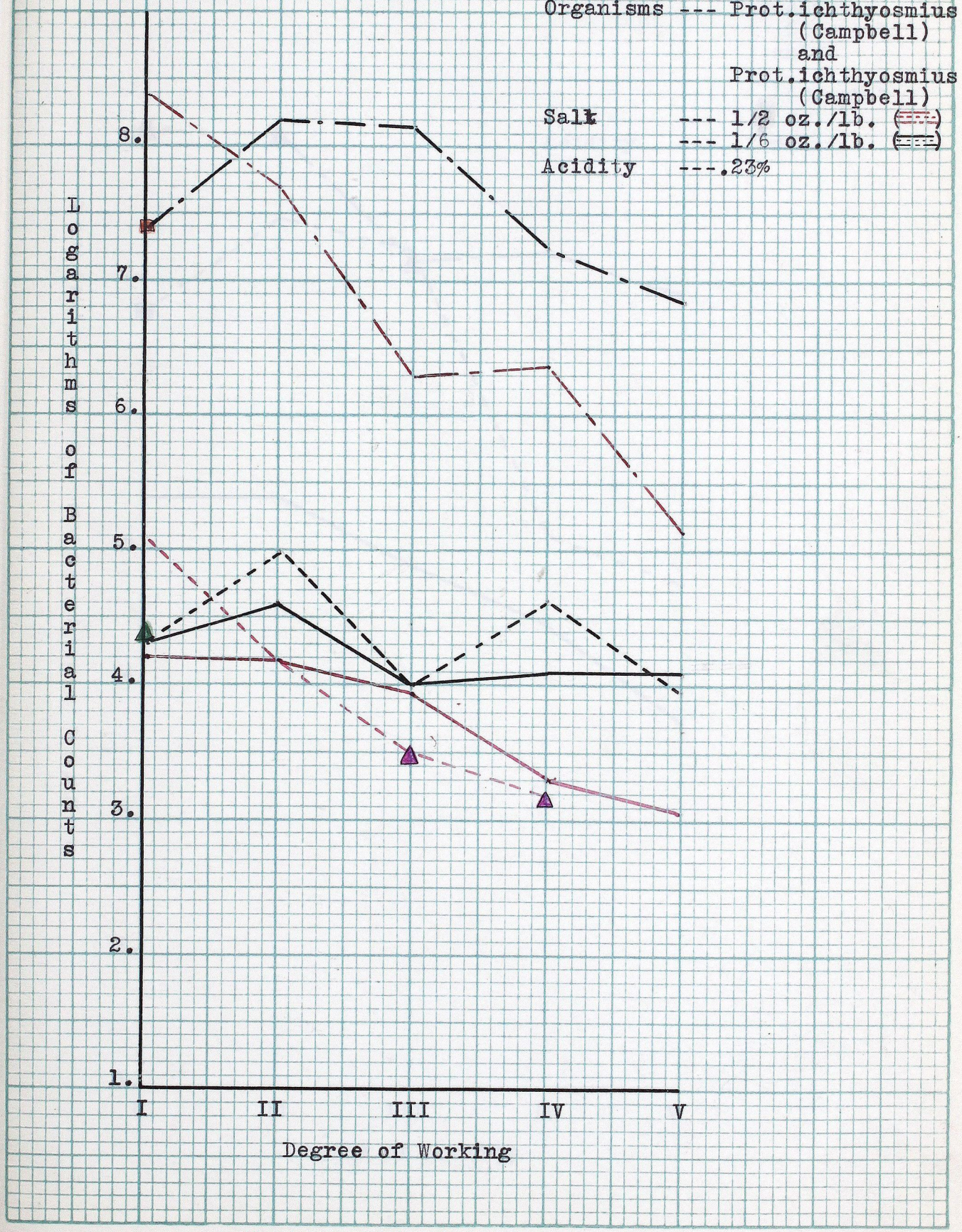
This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.

T.

Fig. 17.

Churning 17.

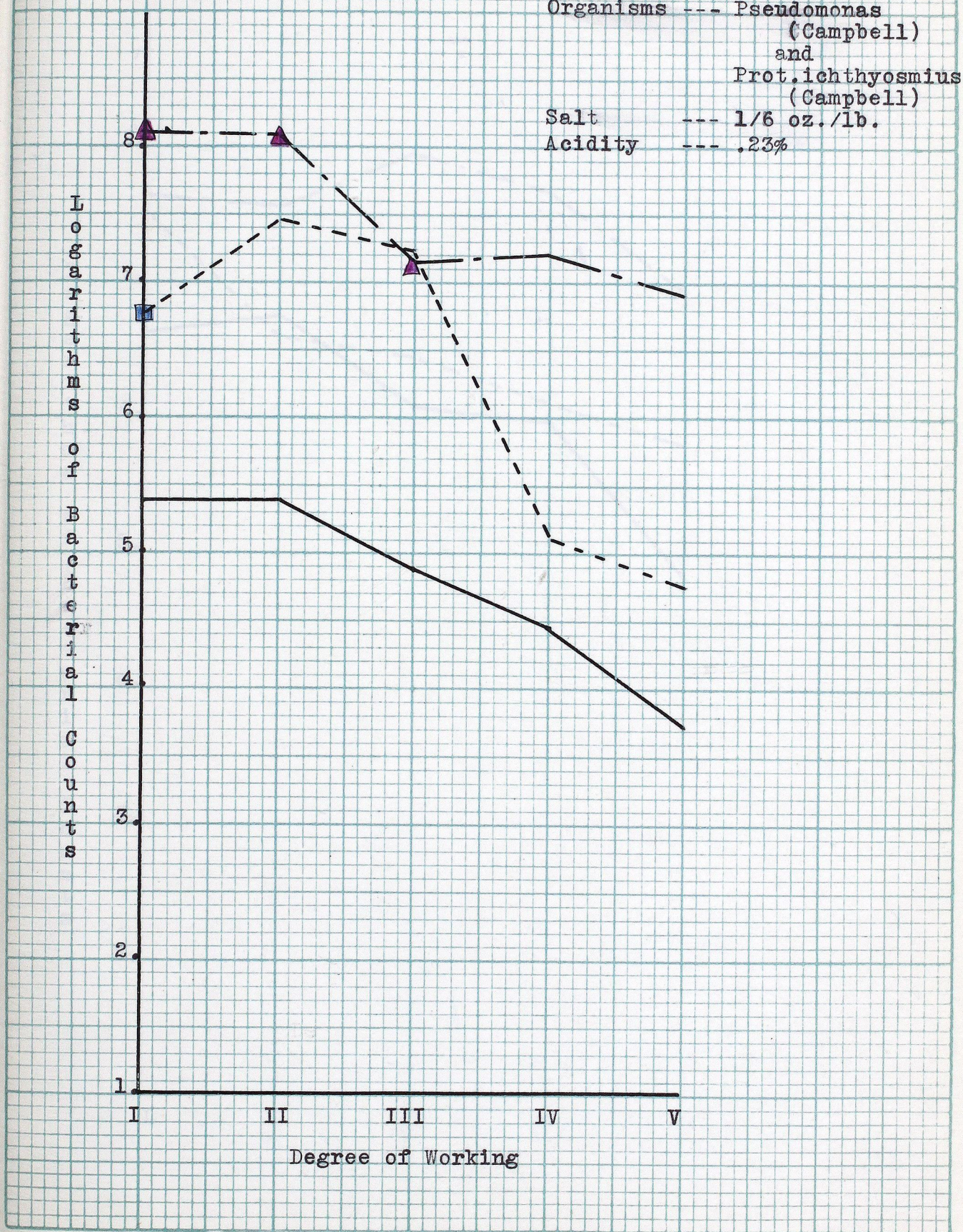


This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.
T.

Fig. 18.

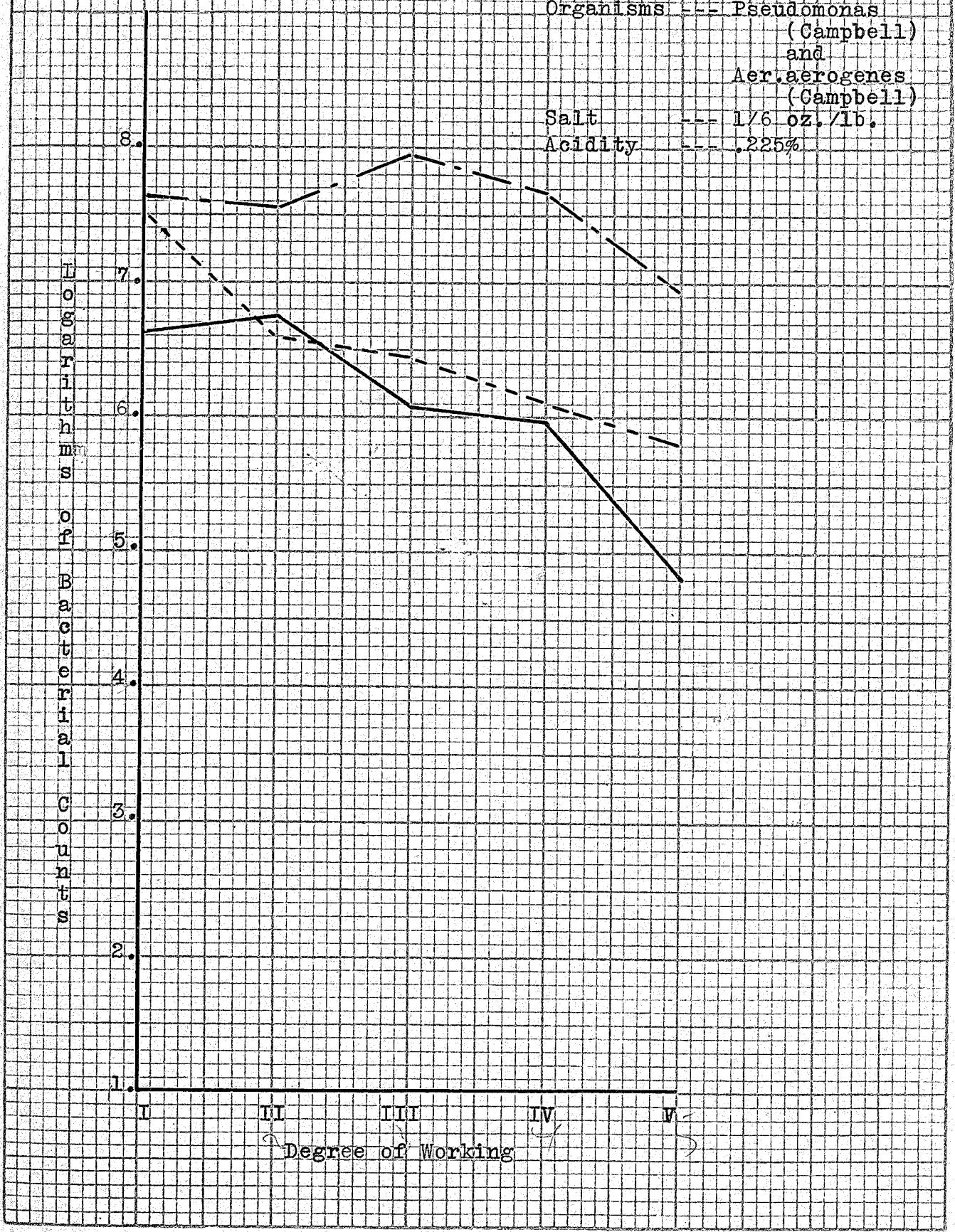
Churning 18.



This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.
T.

Fig. 19. Churning 19.

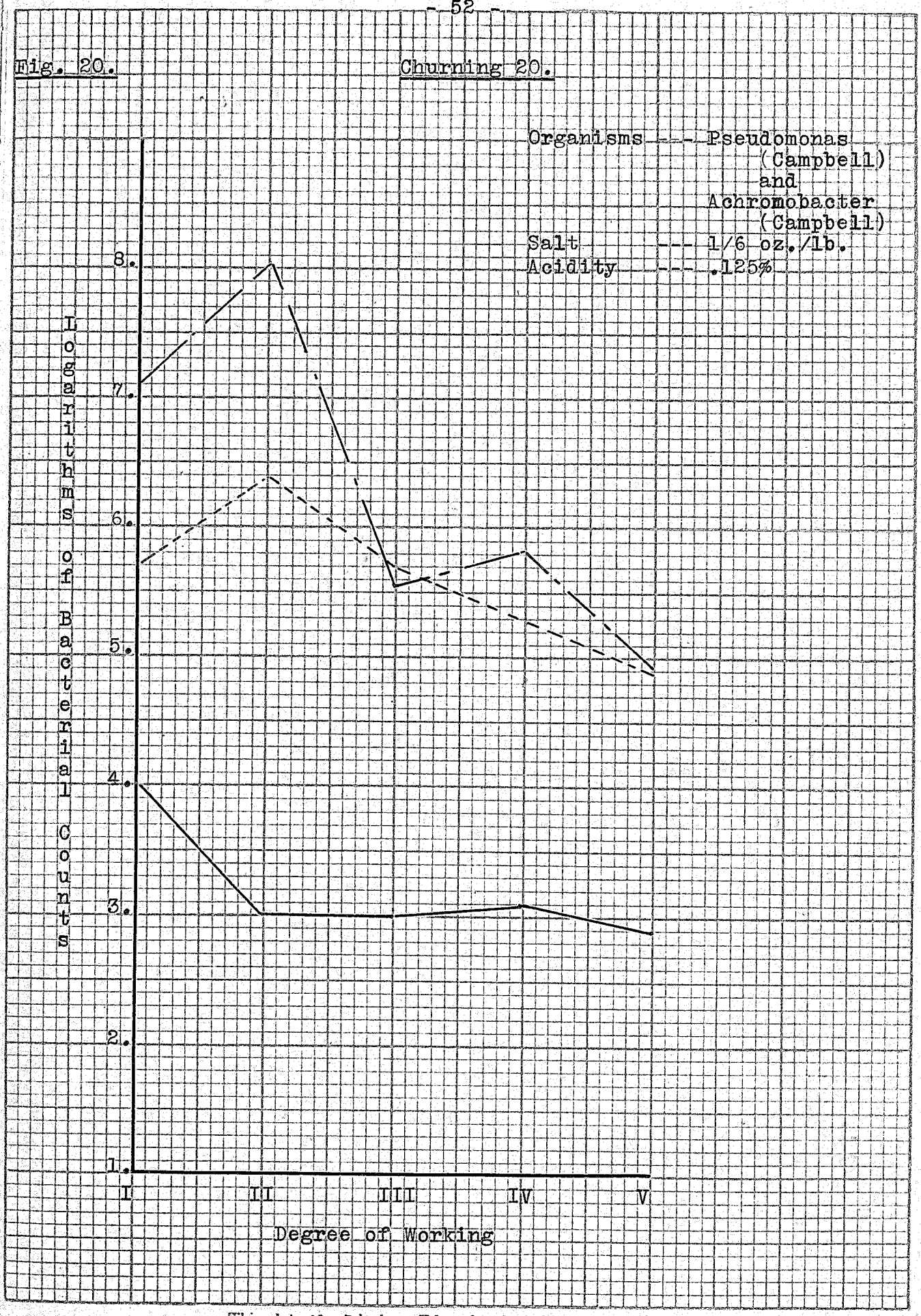


This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.

T.

Fig. 20. Churning 20.



This plate 10 x 7 inches. Edge of each small square 1/10 inch.

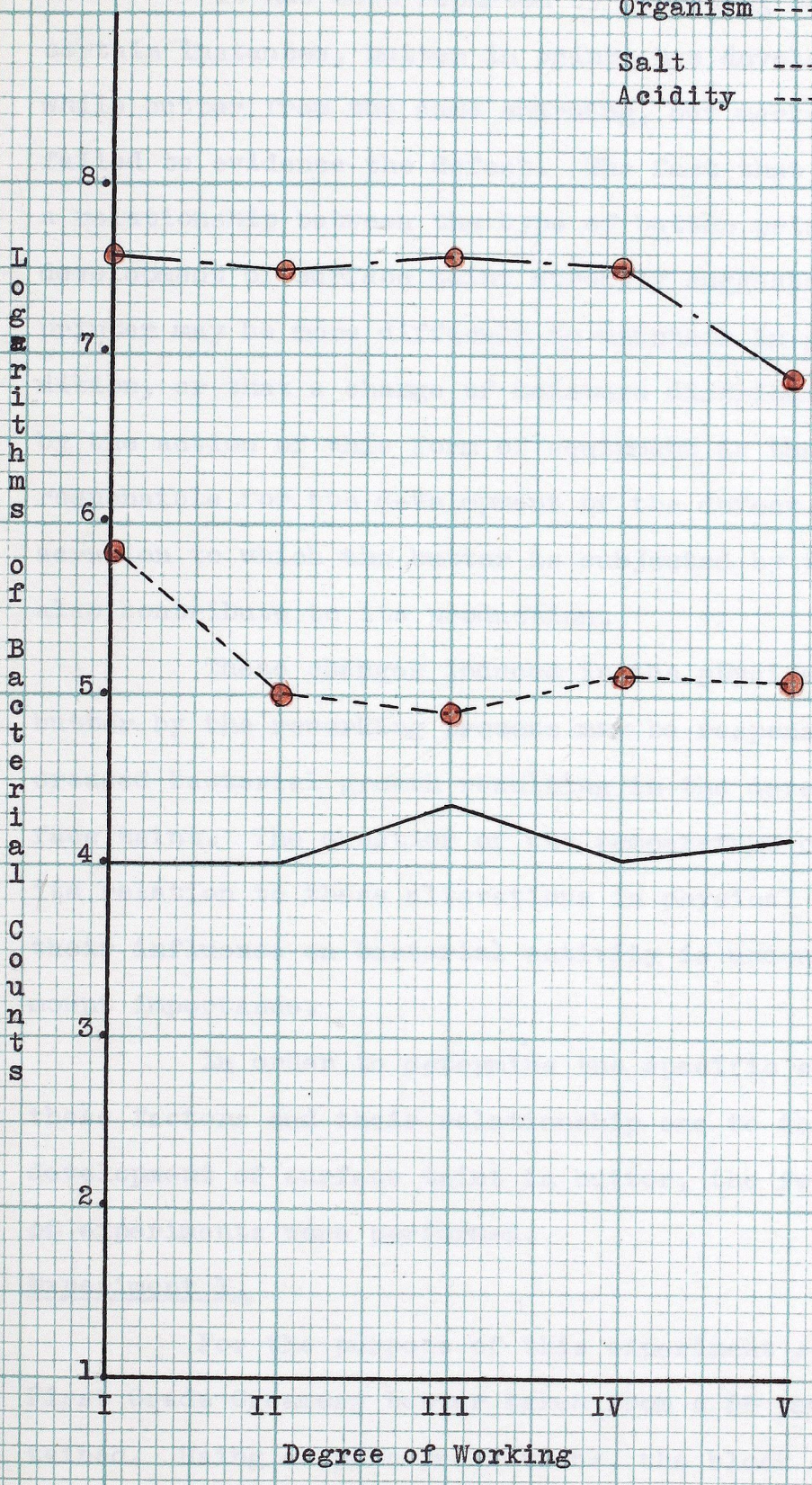
C.C.C.

T.

Fig. 21.

Churning 21.

Organism --- Ps. putrefaciens (Hammer)
Salt --- 1/6 oz./lb.
Acidity --- .125%



This plate 10 x 7 inches. Edge of each small square 1/10 inch.

C.C.C.
T.

PART II B - Surface Taint in Commercial Butters.

It has been reported by marketing organizations that in certain instances, butter in prints has developed surface taint, while butter from the same churning, but marketed in boxes has failed to evidence the defect. The fact that there seems to be a relationship between the type of butter printer used and the development of surface taint strongly suggests that this type of printer may be more difficult to maintain in good sanitary condition, or that a change of moisture distribution brought about by the action of this form of equipment is the factor mainly responsible for the development of the defect. The degree of aeration to which the butter is subjected by this process may also contribute to its production.

These changes induced in the physical nature of the butter by the reworking process may be responsible for an increased bacterial growth resulting in the development of surface taint. The question of the temperature of the butter in its relation to these alterations in physical structure, and their influence on bacterial activity is undoubtedly of paramount importance.

In order to determine the specific effect of each of these factors and their relationship one to the other on the development of surface taint in butter, the following series of experiments were performed.

Experiment I.

For the purpose of this experiment several pounds of First Grade Creamery butter taken from a 56 pound box were obtained. Using aseptic precautions, the block of butter was

divided into two equal sized parts, confining the entire exposed surfaces to one portion. These portions are designated as "Surface" and "Interior". Each of these portions was then divided into two parts. One of each of the Surface and Interior portions was then put through a sterile meat grinder, simulating the conditions encountered at times in the commercial printing of butter. Samples of the reworked butter, designated as Surface Grinder and Interior Grinder were then taken. The remaining half of each portion was sampled directly. In all, nine samples of each of the four portions - Surface, Surface Grinder, Interior and Interior Grinder - were taken, employing sterile triers. One sample of each portion was plated immediately on Standard and Malt Agar.

The method adopted for the storage of the remaining samples was designed with the object of providing varying degrees of oxygen supply at different temperatures. A variation in the oxygen supply was obtained by preparing samples in the following manner:

1. Containers filled with packed butter.
2. Containers half-filled with packed butter.
3. Containers half-filled with unpacked butter.
4. Containers half-filled with packed butter and deprived of oxygen by placing in an oxygen free atmosphere. Pyrogallic acid was employed for this purpose.

Duplicate samples of each variation were prepared, one sample being held at 5°C. and the other at 23°C. for a period of seven days. They were then plated on Standard Agar and on Malt Agar. The counts obtained are recorded in Table 2.

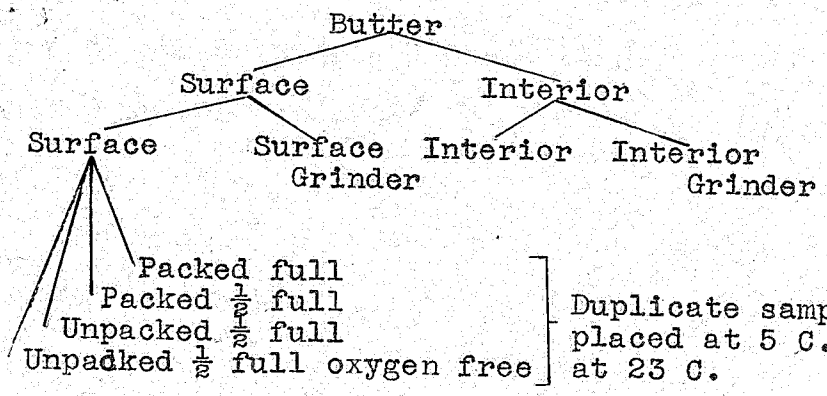
Experiment 1.

Table 2.

Conditions of Storage	Examined Immediately			Examined after 7 days at 5°C.				Examined after 7 days at 23°C.			
	Yeast & Mould	Count on S. A.		Defect	Yeast & Mould	Count on S.A.		Defect	Yeast & Mould	Count on S.A.	
		Total	Prot.			Total	Prot.			Total	Prot.
Surface	10	4,000	0								
Packed Full					10	10,000	0		6,970	760,000	230,000
Packed ½ Full				Unclean	40	190,000	100,000	Unclean	505	540,000	40,000
Unpacked				S.T.	135	25,000	5,000	Stale	1,675	580,000	0
Unpacked O_2 free					10	0	0		650	280,000	10,000
Surface Grinder	275	34,500	8,500								
Packed Full					1,330	320,000	20,000		27,800	2,880,000	430,000
Packed ½ Full					690	110,000	30,000	Unclean	41,500	3,700,000	500,000
Unpacked				S.T.	3,100	85,000	5,000	S.T.	57,000	3,000,000	265,000
Unpacked O_2 free					2,920	10,000	0		41,000	1,440,000	130,000
Interior	60	25,500	1,500								
Packed Full					80	10,000	0		6,680	2,900,000	260,000
Packed ½ Full				Unclean	1,560	10,000	5,000	Unclean	2,280	1,540,000	165,000
Unpacked				S.T.	45	40,000	15,000	S.T.	960	1,870,000	45,000
Unpacked O_2 free					130	90,000	50,000		810	490,000	100,000
Interior Grinder	890	25,000	6,500								
Packed Full				Stale	950	20,000	0		115,200	2,070,000	0
Packed ½ Full				Stale	490	15,000	0	Unclean	45,000	630,000	15,000
Unpacked				S.T.	280	15,000	5,000	S.T.	39,000	2,770,000	205,000
Unpacked O_2 free					370	100,000	20,000		23,300	4,110,000	400,000

* The counts recorded represent the average of plates made from two dilutions.

Experimental Procedure



Discussion

The results recorded in Table 2 for the butters plated immediately after sampling show clearly that the butter employed was of low bacterial content. The figures for the total counts on the Surface and Interior samples indicate that the distribution of bacteria in butter is not uniform and confirm the findings reported by Hammer (10). The total counts obtained on the "Surface", and "Surface Grinder" samples show that the methods used in the sampling and handling of butter for total bacterial count determinations may be of paramount importance. The only difference between the two samples is that prior to plating, the Surface Grinder sample had been subjected to a grinding process. The explanation of the phenomenon may be simply that this process results in a wide distribution of organisms throughout the butter, resulting in an increased count on the aliquot taken for bacteriological analysis in the case of the Surface Grinder sample. The increase in count, however, may only be an apparent one, the lower count obtained on the butter not exposed to grinding being due to the peculiarities in the growth requirements of the bacteria present in

the butter, with resultant failure to develop colonies on plates. The failure to initiate growth on media in the case of the unground butter may be dependent upon the extent of aeration to which the butter is subjected during the grinding process.

The grinding process appears to be without influence on butter taken from the interior of the block so far as the number of bacteria, as revealed by the Plate Method, are concerned, Table 2. However, the distribution of bacteria with respect to species would appear to be altered by the process. Proteolytic types constituted a much larger proportion of the total numbers of bacteria appearing on Standard Agar plates. This finding is also to be seen when the results of the Surface and Surface Grinder samples are observed. Although the Surface sample showed no proteolytic types, these made up one quarter of the total count of the Surface Grinder butter.

The influence of the simple process of grinding on the micro-biological analysis of butter is strikingly revealed when the yeast and mould counts on the four butter samples are considered, Table 2. The grinding of butter, whether from the surface or the interior of a block, results in a marked increase in yeast and mould count. Whatever the explanation of this phenomenon may prove to be, the finding is of significance in so far as the determination of the yeast and mould count in butter is concerned. This method is an accepted standard for the evaluation of butter quality. The findings reported herein indicate that further study should be made of the methods used for the taking and preparing of samples for yeast and mould count determinations.

Although errors inherent in the Plate count of butter

make difficult the interpretation of results, the findings recorded in Table I, show clearly the influence of the grinding process on the bacterial population of butters stored at 5°C and 23°C.

At 23°C. the total count is in every case many times that found at 5°C. and is conspicuously evident in the case of the samples obtained from the interior of the block of butter. Whilst the count obtained on these butters held at 23°C. is as high if not higher than the corresponding surface samples, the counts on the samples stored at 5°C. are consistently low in the case of the Interior Samples. Grinding would appear to have exerted no influence on the bacterial development in Interior butter. It will be recalled that grinding was without influence on the bacterial count of this butter when plated immediately after processing. On the other hand, however, the grinding process is seen to exert a marked effect on bacterial multiplication in butter taken from the surface and held at 23°C., a difference recorded in the case of these samples when plated immediately after sampling. The influence of the grinding process and temperatures of storage on the growth of yeasts and moulds is clearly shown in Table 2.

No specific relationship between bacterial count and the incidence of surface taint as dependent upon the grinding process, is found to exist, Table 2. Although the butter was of high quality, it is to be seen that surface taint developed in seven of the experimental samples. The majority of the remaining samples retained their original quality. Certain of the samples evidenced other defects which are recorded in Table 2. It is to be especially noted that surface taint occurred only

in the unpacked samples, and developed after storage at both 5°C. and 23°C. Other defects were confined practically to containers half-filled with packed butter. In the case of the particular butter used in the experiment, the development of surface taint appeared not to be conditioned by the grinding process, but to depend in great measure on the procedure adopted for the storage of the samples.

It would appear that aeration is a determining factor for the occurrence of surface taint. Apparently bacterial growth of itself does not necessarily account for the development of the defect. The occurrence of surface taint appears to depend on a number of closely integrated biological, chemical, and physical factors. From these experiments it would appear that "aeration" acts as a trigger, setting in motion the chain of events which ultimately results in the development of surface taint, providdd the stage for its development has been set.

Experiments 2 and 3.

The experiment reported upon above was repeated on two subsequent occasions with slight changes in experimental procedure. In the light of the results obtained in Experiment 1, the method adopted for the storage of samples was restricted to unpacked butter in half-filled containers at 5° and 23°C. In addition to the media employed in Experiment 1, all samples were plated on Purple Lactose Agar in an attempt to differentate further the types of microorganisms present under the different experimental conditions.

In Experiment 2, the butter employed was obtained from a churning of butter which, although placed in First Grade on

the wholesale market, developed surface taint when held in prints under retail conditions. The butter for experimental purposes was taken from a 56 pound box of this churning held in wholesale cold storage.

The butter employed in Experiment 3 was also of First Grade; no report of surface taint development in prints on the retail market was reported.

Discussion.

The results recorded in Table 3 show clearly that from a bacteriological point of view these experimental butters were of lower quality than the butter employed in Experiment 1. The butter employed in Experiment 2 was much higher in total count than that employed in Experiment 3. Butter 3 as judged by Yeast and Mould Count would be considered the least desirable of the three samples from the view point of keeping quality, although its bacterial content was not as high as that of Butter 2.

The influence of grinding on butter as recorded by the counts obtained immediately after sampling differ from that reported in the case of Experiment 1. In the second experiment grinding results in a decreased total count particularly on the samples from the Interior of the block. The count obtained on Purple Lactose Agar parallel those obtained on Standard Agar, although at a lower figure. In this experiment, grinding appears to be without influence on bacterial count, using either type of medium. In this instance the counts obtained on the respective media are practically identical.

The findings recorded after incubation for a period of seven days at 5° and 23°C. respectively are difficult to interpret. Although Butter 2 was considered higher in initial total

bacterial counts than Butter 1, the samples taken after storage at both 5° and 23° C showed marked diminution in count, a finding at variance with that obtained in Experiment 1.

Considerable bacterial growth is to be seen in the case of Butter 3, Table 3, particularly in the samples taken from the Interior of the butter and held at 5 C. The data obtained for the changes in bacterial types on storage as revealed by plating on Purple Lactose Agar are of considerable interest.

In Experiment 2, it is to be noted that no alkali producing colonies were indicated by this medium in the case of the butters examined immediately after sampling. After storage, alkali producing organisms are seen to constitute a considerable proportion of the bacterial types appearing in this medium, particularly in the sample held at 23 C. Their absence from the initial plating of the butter may be due to their presence in insufficient numbers in the butter at that time, to be detected in the dilutions employed. The possibility that some condition of the butter prevented initiation of their growth when plated cannot be excluded from consideration as a factor responsible for their apparent absence. It will be recalled that in the case of Experiment 1, aeration was advanced as a possible explanation for the initiation of growth and development of colonies. Although the aeration of grinding was apparently without influence in Butter 2, the appearance of these types on Purple Lactose Agar after storage of the butter may well be explained by the aeration to which the butters are subjected during the holding period. Other factors of course

Table 3

Butter Samples.	Examined Immediately					Examined after 7 days at 5°C.					Examined after 7 days at 23°C.				
Exp. 2	Yeast & Mould	Count on S. A.		Count on P.L.A.		Defect	Yeast & Mould	Count on S. A.		Count on P.L.A.		Defect	Yeast & Mould	Count on S. A.		Count on P.L.A.	
		Total	Prot.	Total	Alk.			Total	Prot.	Total	Alk.			Total	Prot.	Total	Alk.
Surface	30	378,000	2,000	240,000	all acid	S.T.	45	152,000	29,500	168,000	22,000	---	410	69,000	1,500	48,000	44,000
Surface Grinder	60	316,000	500	265,000	all acid	S.T.	50	155,250	5,500	82,000	31,000	---	18,000	160,750	5,000	159,000	150,000
Interior	40	504,000	2,000	375,000	all acid	S.T.	25	98,750	1,000	77,500	12,000	---	370	101,500	2,000	95,500	90,000
Interior Grinder	20	270,000	2,000	213,000	all acid	S.T.	55	77,000	3,500	61,000	10,000	---	8,350	124,500	1,500	121,000	115,000
Exp. 3																	
Surface	6,750	153,500	10,000	153,000	110,000	S.T.	12,550	371,300	4,000	358,000	267,000	---	62,500	720,000	2,000	605,000	480,000
Surface Grinder	8,150	155,500	17,000	143,000	140,000	S.T.	11,725	344,200	90,000	310,000	260,000	---	208,000	134,000	25,000	1,100,000	730,000
Interior	8,100	145,200	1,850	139,000	94,000	S.T.	12,475	2,705,000	20,000	1,800,000	600,000	---	26,500	620,000	2,500	617,000	231,000
Interior Grinder	9,500	141,500	1,600	137,000	100,000	S.T.	110,000	1,000,000	140,000	705,000	650,000	---	21,000	657,500	2,000	545,000	250,000

may be at work. These organisms are peculiarly fastidious with respect to initiation of growth on artificial media. No adequate explanation has so far been advanced for this phenomenon.

Unlike the results obtained in Butter 2, the initial platings of Butter 3 on Purple Lactose Agar revealed a high proportion of alkali producing types which are seen to constitute in large measure the majority of the colonies developing on this medium from the samples stored for seven days at either 5° or 23°C.

A finding of interest, yet difficult to explain, is the occurrence of a high count made up chiefly of acid-forming colonies in the case of the Unground Butter taken from the interior of the block and stored at 5°C.

The growth of yeasts and moulds was quite pronounced in the case of Butter 3 at both temperatures of storage. In the case of Butter 2, which showed a much lower initial yeast and mould count, multiplication was only apparent in the case of samples held at 23°C., and is particularly to be seen in the case of samples submitted to the Grinding Process.

The incidence of surface taint is especially significant, particularly with respect to the question of the temperature to be employed for the storage of butter and with respect to the procedure to be adopted in the storage of samples used to detect the occurrence of the defect. In both Experiment 2 and 3, all butter samples held at 5°C. developed surface taint. No defects were observed in samples held at 23 C. This finding serves to confirm the conclusion drawn from Experiment 1 that "the occurrence of surface taint appears to depend on a number of closely integrated biological, chemical and physical factors".

The fact that surface taint developed in all samples held at 5°C. confirms the finding reported in Experiment 1, that, under the specific experimental conditions adopted in this part of the work, the development of surface taint appeared not to depend on the grinding process. One cannot conclude from these experiments that aeration due to grinding is without influence in the development of surface taint in the printing of butter using certain types of commercial equipment. Work done for a commercial firm experiencing difficulty in the printing of their butter showed that this factor was of decisive importance under the conditions employed by the creamery in question. In this instance 23°C. did not inhibit the development of surface taint.

The failure to demonstrate the critical influence of the grinding process on the development of surface taint experimentally may have depended on an unfortunate or unwise selection of butter for experimental purposes. Any error in this direction was of course unintentional. It is evident that in these butters the stage had been previously set for the production of the defect. Apparently the only factor required was the setting of the trigger, the keeping of the butter at the temperature suitable for its development.

The temperature employed by the Grading Service for the development and detection of flavour defects, and particularly for surface taint, is approximately 23°C. The results reported in this study show clearly that this temperature is unsuitable for the development of surface taint in certain types of butter and that both 5°C and 23°C. should be employed in order to ensure the detection of surface taint in butter cap-

able of exhibiting the defect.

Experiment 4.

The question of the direct contamination of butter with surface taint producing bacteria as a significant factor in the development of surface taint in butter printed from certain types of equipment merits consideration, for in this type of printer, maintenance of proper sanitary conditions is difficult. In attempts to produce surface taint experimentally by direct inoculation of surface taint butter into a normal product, Hammer and Derby (10) were regularly unsuccessful. Shutt (33), on the other hand, succeeded in reproducing "surface flavour" in sterile butter inoculated with Pseudomonas fluorescens. Reference to the exact method of contamination used by him is not given.

In order to determine whether direct contamination of high quality butter with surface taint producing microorganisms results in the development of the defect, the following experiments involving the direct smearing of butter with an actively growing culture of the specific microorganism were carried out. The influence of changes in the physical nature of butter -- particularly the influence of reworking -- on the development of the defect under these conditions of contamination were also studied.

For the purpose of this experiment, several pounds of butter of exceptionally high quality taken from a 56 pound box were obtained. Only butter from the interior of the block was employed. Using aseptic precautions, the block of butter was divided into three equal portions. One portion - Portion 1 -

served as the control. Portion 2 was inoculated with Proteus ichthyosmius (Campbell). Portion 3 was inoculated with a species of Pseudomonas (Campbell). Each portion was then divided into two parts, one of which was sampled directly and the other put through a sterile meat grinder, simulating the conditions encountered at times in the commercial printing of butter, prior to sampling. Triplicate samples of each of the resulting six parts were then taken, one of the three for immediate plating, and the others for storage at 5° C. and 23° C. respectively, prior to plating. on Standard Agar, Malt Agar, and Purple Lactose Agar were employed for the microbiological analysis of the samples. The results of the determinations are given in Table 4.

Discussion.

The high quality of the butter employed in this experiment as based on Grading Standards is confirmed by the results of microbiological analysis as shown in Table 4.

The effect of grinding on the bacterial count of the Control Butter is similar to that obtained in the case of Experiment 1.

The increased bacterial count obtained in the case of the Control Butter when subjected to grinding confirms the results reported in Experiment 1. Whilst a similar effect is to be seen in the case of the portion inoculated with Prot. ichthyosmius, the increased count due simply to the grinding process is not to be seen in the case of the butter contaminated with Pseudomonas putrefaciens. This finding is similar to that recorded in the case of the butters used in Experiments

Table 4.

Exp. 4	Examined Immediately					Examined after 7 days at 5°C.						Examined after 7 days at 23°C.					
	Yeast & Mould	Count on S.A.		Count on P.L.A.	Alk.	Defect	Yeast & Mould	Count on S.A.		Count on P.L.A.	Alk.	Defect	Yeast & Mould	Count on S.A.		Count on P.L.A.	Alk.
		Total	Prot.					Total	Prot.					Total	Prot.		
Butter (control)	0	633	10	50	0	---	0	2,100		1,325	1,325	---	0	26,000	6,000	17,500	13,000
Butter Grinder (control)	0	38,700	3,000	35,500	35,500	---	0	22,500		19,250	1,325	---	0	30,000	11,000	14,300	12,000
Butter inoculated with Prot. ichthyosmius (C.)	2,300	66,000	60,000	94,000	94,000	Definite S.T.	0	34,400,000		65,900,000	65,900,000	Rancid	30	75,400	75,400	63,800,000	63,800,000
Butter Grinder inoculated with Prot. ichthyosmius (C.)	0	439,000	400,000	484,500	484,500	Definite S.T.	0	2,000,000	2,000,000	2,570,000	2,570,000	S.T.	0	6,150,000	6,000,000	5,840,000	5,840,000
Butter inoculated with Pseudomonas (C.)	0	500,000	250,000	580,000	580,000	Modified S.T.	0	1,535,000	600,000	1,532,000	1,532,000	Rancid	0	1,335,000	300,000	945,000	150,000
Butter Grinder inoculated with Pseudomonas (C.)	400	430,000	180,000	775,000	775,000	Modified S.T.	0	600,000	300,000	517,500	517,500	Unclean	0	650,000	300,000	385,000	288,000

2 and 3, which failed to show an increased count on grinding. It may well be that the specific type of microorganism predominating in a butter may determine whether or not an increased count in butter results from the physical changes induced by grinding.

The apparent absence of alkali-forming microorganisms on plates made from the Control Unground Butter, and their predominance on plates made from the same butter subjected to grinding is a finding of considerable importance. Whatever the explanation, grinding results in the appearance on plates of microorganisms which had previously failed to develop. The suggestion made in explanation of the findings recorded on Experiment 1, that aeration may be an important factor in this connection, is thus rendered more plausible.

Neither of the Control Butters held at 5° or 23°C. showed a marked increase in bacterial count when compared with the results obtained in the case of the infected butters. Microbial growth in the samples inoculated with Proteus ichthyosmius was indeed marked.

Both Control Butters maintained their original quality in so far as flavour is concerned, when stored at either 5° or 23°C. All the contaminated samples developed defects. Surface taint was found in all the samples held at 5°C. and occurred in the case of the butter inoculated with Proteus ichthyosmius and submitted to the grinding process at 23°C. The other samples held at this temperature were graded rancid or unclean. Rancidity often develops as an aftermath of surface taint.

The results of this experiment show clearly that

direct contamination of butter by smearing with surface taint producing bacteria results in the development of the defect. Multiplication of these organisms in butter has also been demonstrated. The inadvisability of storing butter at 5°C. as a preventive of surface taint development is again confirmed.

PART II C. - Experimental Churnings(1941)

Experimental.

Because of the difficulty encountered in Part II A in successfully and consistently producing surface taint experimentally, attempts were made to discover the conditions unwittingly set up in previous work, which had exerted an inhibitory effect on the development of the defect. Experiments designed to determine the influence of the nature of the medium employed for growth of the organisms used to inoculate the butter, the source of the cream supply, and the method of infection were undertaken with this object in view. The influence of acidity and neutralization on the development of surface taint was re-investigated.

In this series of experimental churnings the procedure adopted in the work reported under Part II A was in large measure followed. Samples, however, were taken at three stages of working only, as follows:-

I. Slightly worked.

II. Underworked.

III. Thoroughly worked.

Bacteriological examinations of the butter samples were not carried out.

The microorganisms used in this part of the work were Ps. putrefaciens (Hammer), Prot. ichthyosmius (Hammer), Prot. ichthyosmius (Campbell), a species of Pseudomonas (Campbell), and two strains of Ps. putrefaciens recently isolated from surface taint butter produced in British Columbia.

Influence of Medium for Growth of Inoculum on Development of Surface Taint.

In the light of work done on the influence of the nature of the medium - particularly with respect to nitrogen and carbon supply - on the metabolism of the surface taint bacteria, and reported upon in Part III of this thesis, investigation of the effect of the nature of the medium used for the growth of the organism employed in experiments on surface taint production in experimental churnings was undertaken. The media employed were:-

Tryptic Casein (Commercial) Digest Broth (Cole) (7) — T.C.D.B.

Davis Broth (Davis) (9)

Tryptic Casein (purified) Digest Broth

Tryptic Casein (Purified) Digest Broth + 0.5% Glucose.

Mode of Infection.

In conjunction with the investigations on the influence of the type of medium on surface taint development, experimental churnings were carried out in order to determine whether or not the mode of infection of the butter with the surface taint organism was an important factor governing the development of the defect.

Prior to pasteurization, the cream was divided into two equal portions. The usual method of inoculation via the washwater as practised under Part II A was employed in the case of one portion. Contamination of the second portion was effected by inoculation of the cream one half hour prior to churning.

Discussion.

As may be seen in the results of Experiments 1 - 7,

surface taint was produced in the majority of cases irrespective of the medium employed for growth of the inoculum, Tables 5 - 8. It appears that both Davis and Tryptic Broths favour the development of surface taint in butter. Therefore it is to be concluded that the nature of the nitrogen source as present in the two different media, has no influence on the production of the defect.

The results of Experiments 6 and 7 show clearly that the presence of glucose in the medium used for growth of the organism does not inhibit the development of surface taint in butter.

The method of inoculation as studied in Experiments 1, 2, and 3 also appeared to have little effect on the production of the taint. Except in Experiment 1, there was no marked difference in the development of surface taint when either of the two methods of infection were employed. In Experiment 1, inoculation of the cream appeared to favour the development of surface taint.

In this series of experiments, the degree of working had little influence on the development of surface taint, Tables 5 - 8.

In Experiment 4, using Prot. ichthyosmius (Campbell) the butters held at 5°C. for a week, and then raised to 23°C., prior to grading, gave a very strong odour of ammonia when the sample jars were first opened. Fifteen minutes later, however, they were regarded as characteristic surface taint butters. This phenomenon may be explained by the supposition that the surface taint was masked by the strong ammoniacal odour but was later revealed, after the more volatile ammonia had escaped,

following the opening of the samples. However, the influence of aeration on the development of the defect, is not to be overlooked. It may well be that the admission of oxygen even at this stage, acts as a trigger, setting off the chain of events leading to the development of the defect. The importance of aeration at all stages in the manufacture and storage of butter in its relation to the development of defects is a problem requiring further investigation.

The results recorded in Tables 5 - 8, offer conclusive evidence that microorganisms other than Ps. putrefaciens are capable of producing the characteristic surface taint defect in butter when inoculated either via the cream or wash water.

The fact that in this series of churnings, no difficulty was encountered in the development of surface taint experimentally shows clearly that failure to obtain surface taint consistently in Part II A was not dependent upon the nature of the medium employed for the growth of the causative organism nor upon the mode of infection.

Source of Cream Supply.

The cream used for Experiments 1 - 7, Cream Source 2, was of different origin than that employed in Part II A, Cream Source 1. Whether or not the source of cream supply was a factor influencing the incidence of surface taint in the experimental churnings was determined by making butter under identical conditions, employing cream from both sources, Experiments 8 and 9, Table 9. Tryptic Casein (Commercial) Digest Broth was used as the medium for growth of the inoculated organism in these and subsequent experimental churnings.

Results.

From the results obtained in Experiments 8 and 9, it can readily be seen that the source of cream supply had some influence, although slight, on the specific nature of the putrid defect developing in butter. With Ps. putrefaciens, there was no apparent effect, but with Prot. ichthyosmius (Campbell) the characteristic surface taint defect occurred only under certain conditions of working and holding in butter made from Cream 1, whereas in the case of butter made from Cream 2, the defect occurred under all experimental conditions.

These findings suggest that the difference in the cream supply may have been responsible in a measure for the failure to produce the characteristic defect in butters made under Part I A.

Acidity and Neutralization.

In order to reinvestigate the specific influence of acidity and neutralization on the production of surface taint, the following experiments were carried out.

Acidity.

Raw sweet cream was divided into three equal parts, pasteurized and cooled. Sterile lactic acid in sufficient quantity to increase the original titratable acidity by 0.1% and 0.2%, respectively, were added to two of the fractions. After being held overnight in the icebox, the creams were churned employing the procedure previously described -- Experiments 10 and 11, Tables 10 and 11.

Neutralization.

In this series of churnings the procedure followed

was similar to that employed in the "Acidity Series", except that the lactic acid was added prior to pasteurization, and the varying degrees of neutralization employed were obtained by the addition of Wyandotte CAS, added at a temperature of 90°F., Experiments 12 and 13, Tables 12 and 13.

Further studies on the influence of acidity and neutralization were undertaken in Experiments 14 and 15, Tables 14 and 15. Raw sweet cream was divided into three portions. One portion served as the control -- Cream 1. The other two portions were acidified to 0.32%, acidity, following in one case the procedure outlined under the "Acidity Series" -- Cream 2, and in the other, the procedure used in the "Neutralization Series" -- Cream 3. Cream 3 was neutralized employing Wyandotte CAS.

Discussion.

Although acidity has been claimed to exert an inhibitory influence on the development of surface taint, the results of Experiments 10 and 11 show clearly that experimental butters made from cream at an acidity as high as 0.3% regularly developed the characteristic defect when the water used for washing the butter was infected with certain surface taint producing bacteria. Contamination with Ps. putrefaciens, Prot. ichthyosmius (Campbell) regularly resulted in its development. At this acidity, Prot. ichthyosmius (Hammer), however, failed to produce the defect.

Neutralization of the Lactic Acid of the cream did not appear to have any influence, neither inhibiting nor stimulating the development of the defect when Prot. ichthyosmius (Campbell)

Ps. putrefaciens (Hammer), and Pseudomonas (Campbell) were used as the contaminating organisms, Tables 12 and 13.

In the case of Prot. ichthyosmius (Hammer), neutralization of the cream did not lead to the development of surface taint, but instead resulted in the development of other equally undesirable defects. Over neutralization of the cream resulted in a neutralizer odour in the case of Prot. ichthyosmius (Campbell). With Ps. Putrefaciens, however, the characteristic surface taint odour was produced.

From this series of experiments it is difficult to draw specific conclusions as to the influence of holding temperature on the development of surface taint. Critical examination of the tables show, however, that there is a greater tendency towards the production of the characteristic surface taint odour in butter held at 5°C., than at 23°C., confirming the findings recorded for commercial butters in Part II B.

The results obtained on experimental butters made from creams inoculated with different species of the Genus Proteus and the Genus Pseudomonas are given in Table 16.

The data presented in Table 16 lend support to the evidence previously given that several species of microorganisms are capable of producing surface taint in butter.

Table 5.

Experiment 1.

Organism	Medium	Mode of Infestion	Degree of Working	Defect At 5°C.	Defect At 23°C
Prot. ichthyosmius (Campbell)	T.C.D.B.	Wash Water	I	--	Potent
			II	--	"
			III	--	"
		Cream	I	S.T.	Rotten
			II	S.T.	Synthetic
			III	S.T.	Potent
Control				Normal	
			Experiment 2.		
Ps.putrefaciens (Hammer)	T.C.D.B.	Wash Water	I	S.T.	Slight S. T.
			II	Putrid	Rancid
			III	--	Synthetic
		Cream	I	Synthetic	--
			II	--	Suggestion of S. T.
			III	Putrid	--
Control				Normal	

Table 6

Experiment 3.

Organism	Medium	Mode of Infection	Degree of Working	Defect At 5°C.	Defect At 23°C.
Prot. ichthyosmius (Hammer	T.C.D.B.	Wash Water	I	S.T.	Amine-like
			II	Strong S.T.	Putrid
			III	S.T.	Slightly fecal
		Cream	I	S.T.	Putrid
			II	S.T.	Putrid
			III	S.T.	S.T.
Control				Normal	

Table 7 Experiment 4 Medium

Organism	Medium	Mode of Infection.	Degree of Working	Defect At 5°C.	Defect At 23°C.
Prot. ichthyosmius (Campbell)	Davis Broth	Wash Water	I	All samples were strongly NH_3 when first opened. S.T. apparent 15 min. later.	S.T.
			II		S.T.
			III		S.T.
Pseudomonas (Campbell)	Davis Broth	Wash Water	I	Slight S.T.	S.T.
			II	Slight S.T.	S.T.
			III	Slight S.T.	S.T.
Control				Normal	
			Experiment 5		
Prot. ichthyosmius (Hammer	Davis Broth	Wash Water	I	--	S.T.
			II	stale, cheesy	rancid
			III	S.T.	S.T.
Ps. putrefaciens (Hammer)	Davis Broth	Wash Water ,	I	S.T.	butyric
			II	S.T.	"
			III	S.T.	"
Control				Normal	

Table 8

Experiment 6.

Organism	Medium	Mode of Infection	Degree of Working	Defect At 5°C.	Defect At 23°C.
Prot. ichthyosmius (Campbell)	T. C. D. B. Wash Water (pure)		I	S.T.	S.T.
			II	S.T.	S.T.
			III	Not S.T.	S.T. cheesines
	T. C. D. B. Wash Water (pure)		I	Slight S.T.	Slight S.T.
	½% glucose		II	S.T.	S.T.
			III	--	Slight S.T.
			Experiment 7.		
Ps. putrefaciens (Hammer)	T. C. D. B. Wash Water (pure)		I	S.T.	S.T. - rancidit
			II	S.T.	S.T.
			III	S.T.	S.T.
	T. C. D. B. (pure)		I	rancid, S.T. previously	S.T. once
	½ % glucose		II	rancid, S.T. Previously	S.T.
			III	rancid, slight S.T.	slight S.T.

Table 9

Experiment 8

Organism	Medium	Mode of Infection	Source of Cream Supply	Degree of Working	Defect At 5°C.	Defect At 23°C.
Prot. ichthyosmius (Campbell)	T.C.D.B.	Wash Water	2.	I	S.T., not quite typical	S.T.
				II	S.T., trace	S.T.
				III	S.T.	S.T.
	T.C.D.B.	Wash Water	1.	I	not S.T.	Putrid
				II	rancid, cheesy	Suggestion of S.T.
				III	S.T.	not S.T.
(The incubation of these samples at 5 C for 5 days brought up S. T. in the C.F. butter, and more strongly in the J.F. butter.)						
Experiment 9.						
Ps. putrefaciens (Hammer)	T.C.D.B.	Wash Water	2.	I	S.T.	S.T.
				II	S.T.	S.T.
				III	S.T.	S.T.
	T.C.D.B.	Wash Water	1.	I	S.T.	S.T.
				II	S.T.	S.T.
				III	S.T.	S.T.
Control					Normal.	

Table 10 Experiment 10.

Organism	Medium	Mode of Infection	Acidity prior to Churning	Degree of Working	Defect At 5°C.	Defect At 23°C.
Prot. ichthyosmius (Campbell)	T.C.D.B.	Wash Water	.125%	I	S.T., very strong	slight S.T.
				II	Suggestion of S.T.	S.T.
				III	--	S.T.
			.225%	I	slight S.T.	slight S.T.
				II	Hint of S.T.	S.T.
				III	"	Putrid
			.31%	I	No S.T.	S.T.
				II	First Stages of S.T.	
				III	S.T.	Stale

#The S.T. which developed in sample I held at 5 C. and then raised to 23 C. was much stronger than that which was produced in the 23 C. samples.

Table 11

Experiment 11.

Organism	Medium	Mode of Infection	Acidity prior to Churning	Degree of Working	Defect At 5°C.	Defect At 23°C.
Ps. putrefaciens (Hammer)	T.C.D.B.	Wash Water	.11 %	I	S. T.	S. T.
				II	S. T.	S. T.
				III	S. T.	Rancid
			.22%	I	S. T.	Suggestion of S.T.
				II	S. T.	S.T.
				III	S. T.	Rancid
			.32%	I	S. T.	Rancid
				II	S. T.	Slight S.T
				III	S. T.	Strong S.T

Table 12

Experiment 12.

Organism	Medium	Mode of Infection	Acidity prior to Neut.	Acidity after Neut.	Degree of Working	Defect At 5 C.	Defect At 23 C.
Prot. ichthyosmius (Campbell)	T.C.D.B.	Wash Water	.105%	.105%	I	S.T.; rancid.	Slight S.T.
					II	S.T. "	S.T.
					III	Rancid	Slight S.T.
			.305%	.135%	I	S.T.	Neut. S.T.
					II	Rancid	Slight S.T.
					III	S.T.	S.T.
			.305%	.09%	I	--	Neut.
					II	Trace of S.T.	S.T.
					III	Synthetic.	Neut.
Control						Stale	

Table 13

Experiment 13

Organism	Medium	Mode of Infection	Acidity prior to Neut.	Acidity after Neut.	Degree of Working	Defect At 5 C.	Defect At 23 C.
Ps. putrefaciens (Hammer)	T.C.D.B.	Wash Water	.125%	.125%	I	S.T.	S.T.
					II	S.T.	Slight S.T
					III	S.T.	Trace of S.T.
			.325%	.125%	I	S.T.	S.T.
					II	S.T., strong	S.T. - slight
					III	S.T.	S.T.
			.325%	.075%	I	S.T., strong	S.T.
					II	S.T.	S.T.-slight
					III	S.T.	S.T.-putrid
		Those butters held at 5 C. for one week and then raised to 23 C., on being judged after 5 days at 23 C. gave a curdy, unclean, or putrid odour when the samples were first opened. Fifteen minutes later, however, all of them evidenced typical Surface Taint.					
Control						Cheesy	

Table 14 Experiment 14

Organism	Medium	Mode of Infection	Acidity prior to Neut.	Acidity after Neut.	Degree of Working	Defect At 5 C.	Defect At 23 C.
Prot. ichthyosmius (Hammer)	T.C.D.B.	Wash Water	.12%	.12%	I	Suggestion of S.T.	Trace of S.T.
					II	"	"
					III	"	"
			.32%	.32%	I	--	Not S.T.
					II	--	Unclean
					III	Acid	Clean
			.32%	.16%	I	Synthetic	Rancid
					II	Unclean	"
					III	Not S.T.	"
Control						Normal	

Table 15

Experiment 15

Organism	Medium	Mode of Infection	Acidity prior to Neut.	Acidity after Neut.	Degree of Working	Defect At 5 C.	Defect At 23 C.
Pseudomonas (Campbell)	T.C.D.B.	Wash Water	.135%	.135%	I	S.T.	S.T.
					II	Neut. later S.T.	S.T.-slight
					III	S.T. - not as much	S.T.
			.335%	.335%	I	Acid- later S.T.	S.T.
					II	Strong S.T.	S.T.-trace
					III	Strong S.T.	S.T.-suggestion
			.335%	.175%	I	S.T.	S.T.
					II	Suggestion of S.T.	S.T.
					III	Strong S.T.	S.T.

* In the case of the butters incubated at 23 C. the defect was not as strong in those of the third degree of working as the samples of the second.

Table 16

Experiment 16

Organism	Medium	Mode of Infection	Degree of Working	Defect	
				At 5° C.	At 23° C.
Prot. ichthyosmius (Hammer)	T.C.D.B.	Wash Water	I	S.T.	S.T.
			II	S.T.	S.T.
			III	S.T.	S.T.
Prot. ichthyosmius (Campbell)	T.C.D.B.	Wash Water	I	--	--
			II	--	S.T.
			III	--	S.T.
Control				Normal	
			Experiment 17		
B 9.	T.C.D.B.	Wash Water	I	S.T.	S.T. very strong
			II	S.T.	S.T. very strong
			III	S.T.	--
B 13.	T.C.D.B.	Wash Water	I	S.T.	S.T. very strong
			II	S.T.	S.T. very strong
			III	S.T.	S.T. very strong
Control				Normal	

PART III - The Chemistry of Surface Taint.

Little definite information as to the nature of the substance produced in butter by bacterial action and responsible for the undesirable odour characteristic of Surface taint, is as yet available. Derby and Hammer (10) state that "Protein decomposition of a fairly definite character is involved in the development of surface taint.", but make no reference as to the specific nature of the degradation. Dunkley (11), in work on the chemistry of surface taint, shows that the "sweaty feet" odour produced in milk by Ps. putrefaciens is volatile with steam from an acidic solution, suggesting that the compound producing this odour is of an acidic nature. He claims that it is a non-nitrogenous compound.

From time to time, as the work reported upon progressed, several hypotheses postulating the possible nature of the odoriferous substances and the mechanism of their formation by the bacterial breakdown of the protein material in butter were advanced. Experiments designed to substantiate or disprove the validity of these hypotheses were performed.

Indole Hypothesis.

Historical.

In work carried out by Campbell (3) on the hypothesis that the odour characteristic of surface taint was due to the presence of amines produced from amino acids by the causative microorganisms, melted butter to which indole and ethylamine hydrochloride had been added was considered by competent graders to be strongly suggestive of the odour of surface taint. In further work employing different combinations of possible

odoriferous substances, butters containing indole as one of the added compounds always were judged as closely resembling surface taint.

In compilation of work on the production of indole by bacteria, Stephenson (36) states that its formation is inhibited by the presence of carbohydrate, glycerol, and calcium lactate. Campbell (4) found that butter made from neutralized cream in which acid had been formed by the Lactic Acid Bacteria failed to develop surface taint, a finding which he interpreted as substantiation of his "Indole Theory" for Surface Taint production in butter. He attributed the inhibition of Surface Taint development as due to the lack of Indole formation which he claimed was due to the presence in the cream of the carbohydrate, lactose, and the calcium lactate, formed on neutralization, acting as inhibitors in the manner described by Stephenson.

Woods (38) reported that the degree of aeration influenced the rate and completeness of the reaction of the quantitative production of indole from tryptophan by thick washed suspensions of Bact. coli. Aeration was found to increase indole formation.

Fildes (14) in studies on the production of indole from tryptophan by Bact. coli using the washed cell technique, found that the suspensions when grown in the presence of tryptophan were about twenty-five times more active than when grown in the absence of tryptophan.

Evans, Handley, and Happold (13), in working on the production of "tryptophanase" (the coupled oxidative deamination and decarboxylation in an enzymic system which is

specific for the tryptophan-indole raction) by Esch.coli found that the presence of tryptophan in a medium is essential for the production of the tryptophanase system. It was found also that the presence of glucose in the growth medium has an inhibitory effect on the formation of the tryptophanase system, thus preventing the production of indole by the cell suspension. However, once the tryptophanase system has been set up by growth in a sugar free tryptophan medium, glucose has no inhibitory effect on the production of indole by the cell suspension.

Experimental.

Prior to the setting up of experiments designed to prove or disprove the Indole Theory for the development of Surface Taint in butter, a study of the sensitivity of various tests employed for the detection of indole and the determination of the indole-producing abilities of the microorganism under ideal conditions for its elaboration in bacteriological media were undertaken.

The sensitivity of Nencki's reagent, the Weyl-Legal reagent, Ehrlich's para-dimethyl-amino-benzaldehyde test, the procedure of Woods (38) and the xylene extraction method of Happold and Hoyle (19) for the detection of indole was determined. The method of Happold and Hoyle gave by far the most sensitive and delicate reaction.

Employing this test, the indole producing abilities of the microorganisms used in this study were determined after growth for varying periods of time in Tryptic Casein Digest Broth - a medium found suitable for indole production. Tests were performed on cultures inoculated at 23°, 30°, 31°C., res-

pectively. The results are shown in Table 17.

Proteus ichthyosmius (Hammer) is seen to give a distinctly positive test at all temperatures. 30° and 37° C., however, appeared more suitable for indole development than 23° C. Ps. putrefaciens and Proteus ichthyosmius (Campbell) both failed to give positive tests, Table 17.

Studies on the inhibitory effect of glucose and Ca-lactate on Indole production were of necessity limited to Proteus ichthyosmius, the only culture giving a positive Indole Test in ideal artificial media.

In order to determine the effect of glucose on the production of indole, experiments were set up whereby Prot. ichthyosmius was inoculated into both T.C.D.B. and Tryptone Broth containing increasing percentages of glucose. Tryptone Broth, a nitrogen source commonly employed for the Indole Test, served as a control for the T.C.D.B. The effect of the presence of glucose in the medium used for the growth of the culture prior to inoculation into the test broths, on Indole production, was determined by growing the organism in T.C.D.B., containing varying percentages of glucose. The results of these determinations are given in Table 18.

Indole production was observed in T.C.D.B. containing 0.3% glucose, but not with 0.57% glucose. In tryptone broth, however, no culture containing more than 0.1 % glucose gave a definitely positive test. Glucose is thus shown to exert an inhibitory influence on indole formation from Tryptophan by Prot. ichthyosmius, confirming the findings of Stephenson on B. coli. The presence of glucose in the medium from which the culture is inoculated for the detection of indole is seen to be

without influence on its production.

The effect of Ca lactate on indole production was determined by inoculating Prot. ichthyosmius into typtone broth containing varying amounts of Ca lactate. It was found that Ca lactate partially inhibited the production of indole at 0.5% concentration, a positive test, however, being obtained in the presence of as high as 2.0% Ca lactate.

Taken as a whole, the studies on Indole production lead by inference to the conclusions that indole is not an essential constituent of the characteristic Surface Taint odour. Although only one of the surface taint producing bacteria could be induced to elaborate indole on artificial media, the possibility that indole formation plays a part in the development of surface taint in butter cannot be entirely dismissed from consideration. The inhibiting effect of both carbohydrate and Ca lactate demonstrated in this experiment not only confirms work done by Stephenson on other microorganisms, but makes impossible the complete dismissal of the Indole Hypothesis as an explanation of surface taint development in butter.

If indole formation is a requisite of surface taint development, its presence should be capable of detection in butter showing the defect. Employing the sensitive reaction of Happold and Hoyle, the sera obtained from those butters described in Part IIC., Experiments 1, 2, 3, & 6, Tables 5, 6, & 8, and exhibiting the defect were tested for the presence of indole. Further, in the case of Experiments 3 and 6, sera from aliquots of the butters taken at the time of manufacture, prior to the development of the defect, were taken, placed in

Table 17.

Organism	Medium	Temperature	Time	Reaction.
Prot. ichthyosmius	T.C.D.B.	23°C.	24 Hrs.	+
		23°C.	48 Hrs.	+ +
		30°C.	24 Hrs.	+ +
		30°C.	48 Hrs.	+ + +
		37°C.	23 Hrs.	+ +
		37°C.	48 Hrs.	+ + +
Ps. putrefaciens (Hammer)	T.C.D.B.	23°C.	24 Hrs.	-
		23°C.	48 Hrs.	-
		30°C.	24 Hrs.	-
		30°C.	48 Hrs.	-
		37°C.	24 Hrs.	-
		37°C.	48 Hrs.	-
Prot. ichthyosmius (Campbell)	T.C.D.B.	23°C.	24 Hrs.	-
		23°C.	48 Hrs.	-
		30°C.	24 Hrs.	-
		30°C.	48 Hrs.	-
		37°C.	24 Hrs.	-
		37°C.	48 Hrs.	-

Table 18.

Organism	Medium	Time	Reaction
Prot. ichthyosmius (Hammer)	Tryptone B.+0.1% glucose	52 Hrs.	+
	" +0.2% "	52 "	(+)?
	" +0.3% "	52 "	-
	" +0.4% "	52 "	-
	" +0.5% "	52 "	-
	Tryptone Broth (Control)		++
Prot. ichthyosmius (Hammer)	T.C.D.B.+0.3% glucose	72 Hrs.	+
	" " " " +0.57% "	72 Hrs.	-
	" " " " +0.85% "	72 Hrs.	-
	T.C.D.B. (Control)		+++
Prot. ichthyosmius (Hammer)	T.C.D.B.+0.3% glucose	52 Hrs.	+++
	" " " " +0.57% "	52 "	+++
	" " " " +0.85% "	52 "	+++
Prot. ichthyosmius (Hammer)	Tryptone B.+0.5% Ca lactate	52 Hrs.	++
	" B.+1.0% " "	52 "	+
	" B.+1.5% " "	52 "	+
	" B.+2. % " "	52 "	+
	Tryptone B. (Control)		+++

sterile tubes and incubated at 5°, 23°, and 30°C. for 7 Days and then tested for indole. In none of these sera was the presence of indole even suspected, thus rendering the Indole Hypothesis practically untenable.

Decarboxylation Hypothesis.

Because experimental evidence did not substantiate the hypothesis that indole was an essential constituent of the characteristic surface taint odour, consideration was given to the possibility that a specific amine or combination of amines, produced by decarboxylation of the amino acids by the causative microorganism, was responsible for the development of the defect.

Historical.

Campbell (3) in studies on the hypothesis that indole formation was the necessary reaction for development of surface taint, reported that different amines in combination with indole, approximated the defect.

Gale (15), in recent work on decarboxylation using the washed cell technique, and thus demonstrating the enzymic nature of the reaction, was able to form agmatine, cadaverine, histamine, and amine butyric acid from their respective amino acids using suspensions of Bact. coli. He found that the decarboxylases for the various amino acids varied in their optimum pH activity values. The lower the pH of the medium within limits the higher the activity of the washed suspension. He found also, that the enzymes are not produced if the organisms are grown in an amino acid free medium.

Gale (16) also found that not only gram negative but also gram positive organisms are capable of decarboxylation.

He showed that a strain of Sc. fecalis had the ability to decarboxylate only one amino acid, forming tyramine from tyrosine. However, it was also capable of producing the amino acid ornithine from arginine. Further he showed that putrescine results from the associative action of Sc. fecalis and B. coli on arginine. The action of these organisms on arginine results in:

(1) Decarboxylation of arginine to agmatine by Bact. coli.

(2) Breakdown of arginine to ornithine by Sc. fecalis.

(3) Decarboxylation of ornithine to putrescine by Bact. coli.

Experimental.

The fact that the evidence obtained by Gale had shown that an acid reaction was essential for decarboxylation with resultant amine formation, lent support to this hypothesis. The demonstration of the associated action of microorganisms in the formation of certain of the amines tended to substantiate this concept, especially when the conditions favourable to the production of surface taint in butter are considered.

Employing the technique described by Gale, decarboxylation experiments were performed, employing both Ps. putrefaciens and Prot. ichthyosmius on the amino acids arginine and histidine. In no case was any agmatine or histidine isolated. Unless the conditions requisite for amine production from amino acids by species of the Genera Pseudomonas and Proteus differ from those essential for amine production by strains of B. coli, it may be concluded that these organisms do not elaborate a decarboxylation enzyme system for these amino acids. The reliability of these findings in the light of the difficulties

accompanying this procedure was confirmed when agmatine was readily isolated from arginine employing B. coli communior. Although decarboxylation of the amino acids histidine and arginine, readily observed in the case of members of the coliform group and for this reason selected for study of the decarboxylase activity of the gram negative surface taint producing bacteria, did not occur, the possibility remains that these organisms may be capable of decarboxylating other amino acids.

Deamination Hypothesis.

Owing to the failure to obtain experimental evidence substantiating either the Indole or Decarboxylation Hypotheses for the production of surface taint, and hypothesis postulating the formation of hydroxy and unsaturated acids from amino acids by deamination was considered as a possible explanation of the nature of the mechanism determining the elaboration of the surface taint odour. The evidence put forth by Dunkley (11) that the odoriferous substance is acidic in nature lends some support to this hypothesis. The work of Sasaki (32) on the conversion of tyrosine into parahydroxy phenyl lactic acid by strains of Prot. vulgaris, and that of Raistrick (30) on the formation of Urocanic acid from histidine by species of the coliform group of bacteria strongly suggests that the gram negative microorganisms associated with surface taint development may be capable of deaminating amino acids, and that one of these acids, formed on deamination, or combinations thereof, may be responsible for the characteristic surface taint odour in butter.

Preliminary experiments designed to determine the validity of this hypothesis has shown that the undertaking of a thorough investigation of this aspect of the problem merits consideration.

Summary and Conclusions.

The literature on the putrefactive deterioration of butter has been reviewed.

Detailed descriptions of the microorganisms employed throughout the study are given.

A new medium for the isolation of surface taint producing bacteria is described.

Experiments on the detection of these organisms by their action in milk are outlined.

A technique for experimental butter making on a laboratory scale was developed.

In determining the influence of the degree of working in experimental butter making, it was observed that surface taint or closely related odours and flavours occurred more often in underworked butter than in well worked butter. There was usually a decrease in potency of the odour with an increase in the degree of working. It was also found that there was a tendency towards a definite decrease in bacterial count as the extent of working increased.

It was found that the associative action of surface taint and acid producing microorganisms resulted in a higher incidence of undesirable odours and flavours than when the surface taint producing organism alone was employed.

Defects closely akin to surface taint were encountered more often in butter of low than in butter of high salt content.

In experiments on the influence of grinding on commercial butters it was found that the methods used in the sampling and handling of butter for total bacterial count determinations

may be of paramount importance. Of equal if not greater significance was the finding that the yeast and mould count was influenced greatly by the method of handling.

The results obtained on different butters suggest that an increase in microbial population resulting from physical changes induced in butter by grinding may be determined by the specific types of microorganisms predominating in the butter.

No specific relationship between bacterial count and the incidence of surface taint as dependent upon the grinding process was found to exist.

Direct contamination of a normal commercial butter resulted in the development of surface taint.

The nature of the nitrogen source of the medium employed for growth of the inoculum as studied in this work was shown to have no influence on the production of surface taint. It was found that the presence of glucose in the medium failed to inhibit development of the defect.

The method of inoculation was found to be without influence on the production of the taint.

The source of the cream supply may influence to a slight extent, the development of surface taint.

0.3% titratable acidity of the cream did not inhibit the production of surface taint in the subsequent butters made therefrom employing a surface taint producing bacterium as contaminant. Neutralization of the cream had neither an inhibitory nor stimulatory effect on the development of the defect.

Organisms other than Ps. putrefaciens have been shown

capable of producing surface taint in butter.

The significance of the temperature selected for the holding of butter prior to grading is emphasized. Reasons for the inadvisability of storing butter at 5°C. as a preventive for surface taint development are presented.

The occurrence of surface taint has been shown to depend on a number of closely integrated biological, chemical and physical factors and the significance of aeration as a determining factor for its occurrence has been demonstrated.

Results of work carried out in an attempt to substantiate the Indole Hypothesis showed that it is practically untenable as an explanation of surface taint in butter.

Evidence is presented suggesting that decarboxylation of amino acids with resultant amine formation is not responsible for the development of surface taint.

Preliminary experiments to determine the validity of the Deamination Hypothesis postulating the formation of hydroxy and unsaturated acids from amino acids by deamination showed that the undertaking of a thorough investigation of this aspect of the problem merits consideration.

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