DOMINION OF CANADA
DEPARTMENT OF AGRICULTURE
DOMINION EXPERIMENTAL FARMS

DIVISION OF BACTERIOLOGY

PROGRESS REPORT OF THE DOMINION AGRICULTURAL BACTERIOLOGIST

A. G. LOCHHEAD, B.A., M.Sc., Ph.D.

FOR THE YEAR 1937

Published by authority of the Hon. JAMES G. GARDINER, Minister of Agriculture,
Ottawa, 1939

DOMINION EXPERIMENTAL FARMS

E. S. ARCHIBALD, B.A., B.S.A., LL.D., D.Sc., *Director.*

DIVISION OF BACTERIOLOGY, 1937

A. G. LOCHHEAD, B.A., M.Sc., Ph.D., Dominion Agricultural **Bacteriologist.**
C. K. JOHNS, B.S.A., M.Sc., Ph.D. (Dairying).
R. H. THEXTON, B.S.A., M.Sc. (Soils).
C. B. TAYLOR, B.S.A., M.Sc., Ph.D. (Soils).
M. I. TIMONIN, B.S.A., M.Sc. (Soils).
A. H. JONES, B.S.A., M.Sc. (Foods).
G. B. LANDERKIN, B.S.A., M.Sc. (Foods, dairying).

E. H. GARRARD, B.S.A., M.Sc., Ontario Agricultural **College, Guelph** (Exchange worker, 1937-38).

TABLE OF CONTENTS

DIVISION OF BACTERIOLOGY

Progress Report for the Year 1937

INTRODUCTION

The present report describes briefly the work and progress of the Division of Bacteriology for the year 1937 and up to March 31, 1938, at which date the Division was transferred from the Experimental Farms Branch to the Science Service, Department of Agriculture. The activities of the Division consist in: (*a*) research in microbiology, conducted chiefly in co-operation with the Experimental Farms, and applied to milk production, soils and soil fertility, foods, feeding stuffs and miscellaneous problems of agricultural production and the utilization of agricultural products; (*b*) direct service to farmers, Experimental Farms and other Departmental Branches in bacteriological analysis, preparation of seed inoculants; (*c*) co-operation in administration of acts and other regulatory services under the Department of Agriculture.

During the period under review research has comprised the greater part of the work of the staff of the Division. This report is concerned mainly with progress in investigations in the various fields indicated.

DAIRY BACTERIOLOGY[1]

THE RELATION BETWEEN BACTERIAL INFECTION AND THE BIOCHEMICAL REACTIONS OF MILK FROM STREPTOCOCCUS-FREE QUARTERS

Chronic contagious mastitis, due to infection with *Streptococcus agalactiae,* is generally recognized as one of the most serious problems in dairying. Since both yield and composition of the milk are adversely affected, there is cause for serious concern on the part of the dairy products manufacturer or processor, as well as the dairy farmer. Diagnosis is not nearly as simple as in the case of other diseases such as bovine tuberculosis; most authorities prefer to rely upon bacteriological methods whereby the causative organism may be demonstrated and identified, but such methods are costly and time-consuming. Consequently, there has been a tendency among those engaged in field work to rely almost entirely upon indirect tests, such as those for catalase (or cell count), chlorides or pH. These tests, because of their cheapness, rapidity and simplicity, have been growing in popularity in many districts, and there is an increasing tendency to condemn animals on the basis of the reaction to one or more of these tests.

While milk from animals infected with *Str. agalactiae* generally reacts to these indirect tests, little attention has been given to the possibility that similar reactions may be obtained from the milk of animals free from streptococcal infection. Reports recently published[2] indicate that such reactions may be much more commonly encountered than has previously been suspected. In view of the growing interest in schemes for the control of chronic streptococcic mastitis, in some of which these indirect tests play a prominent part, the need for caution in the interpretation of results from these tests is evident.

[1] Prepared by C. K. Johns.

[2] Hastings, E. G., and Beach, B. A. J. Agric. Res. 54: 199. 1937. Miller, W. T. North American Vet. 17: 3. March, 1936.

The studies reported here deal with animals free from infection with *Str. agalactiae* or other recognized udder pathogen, as shown by (1) repeated failure to isolate these organisms, and (2) the absence of flakes or clots in the milk, or any other microscopic evidence of inflammation.[1] In view of the variety of findings reported in the literature, it seemed advisable to undertake a detailed study of the possible relationship between bacterial numbers and biochemical values, at the same time noting any special type or types of organisms which appeared to be associated with the abnormal composition of the milk. Such a study was made during the year upon selected animals in the herd at the Central Experimental Farm, Ottawa, and has been extended to include similar selected animals at the University of Wisconsin.

In order to observe the relationship between bacterial numbers and abnormal biochemical reactions, samples were taken from individual quarters *at consecutive milkings* for periods varying from five to forty milkings. This method of study brought to light certain relationships which would not have been observed had samples been taken at less frequent intervals. In many instances marked fluctuations were observed in biochemical values (pH, chlorides and catalase) and in bacterial counts. These fluctuations were frequently of a regular, rhythmic nature; high values for chlorides, catalase, and pH following high counts at the preceding milking (see Fig. 1). In general, counts were highest at the morning milkings and biochemical values at the afternoon milkings, although exceptions were noted.

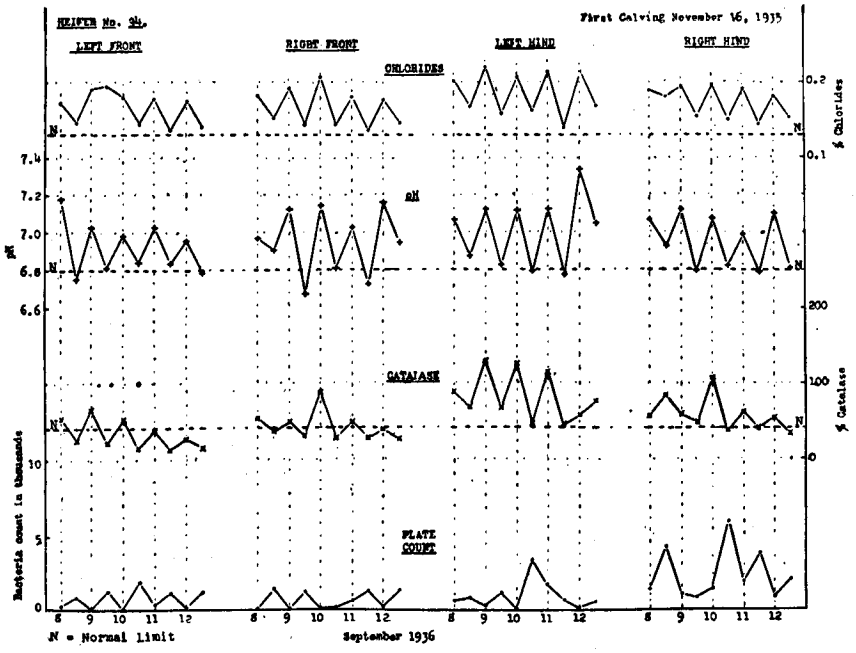

FIG. 1.—Illustrating rhythmic fluctuations in biochemical values and bacterial counts. (Values for afternoon milkings on vertical dotted lines.)

[1] In the case of one heifer (No. 12) over 700 samples of foremilk and strippings were examined with uniformly negative results. Udder pathogens were isolated concurrently from infected animals without difficulty.

Various investigators have put forward different standards for limiting values for chlorides, catalase and pH of normal milk without attempting to determine whether or not these values were truly equivalent. The present studies have shown that there is, with few exceptions, a close correlation between the levels of values for the three tests, all three generally fluctuating in unison. Based upon these findings, tentative standards of 0·13 per cent for chlorides (direct titration of 5 ml. milk diluted with 20 ml. distilled water, using dichlorofluorescein as indicator), 40 per cent for catalase (by the Hastings tube method), and a pH value of 6·8 were adopted.

Of the three tests employed, that for catalase appears to be the most valuable. In addition to its cheapness, ease and simplicity it shows less fluctuation for normal quarters, while the difference between values for normal and abnormal quarters is generally much more marked than that shown by the other two tests. (Fig. 2.) Some evidence was also obtained that it was

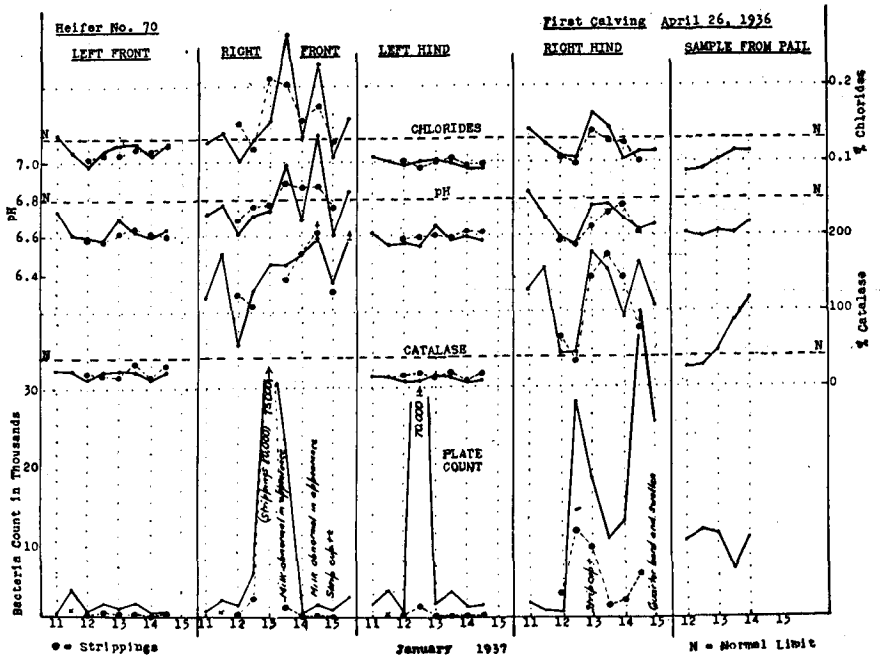

FIG. 2.—Illustrating contrast between normal and abnormal quarters.
(Values for afternoon milkings on vertical dotted lines.)

superior to the cell count in detecting secretion disturbances in quarters free from streptococcic infection, and also to the chloride and pH tests in the early detection of infection with *Str. agalactiae.*

These studies failed to elicit any clear-cut evidence that these abnormal biochemical reactions were due to bacterial infection. No types of organisms were found associated with abnormal reactions which were not also present in milk from normal quarters. A variety of methods was employed in attempts to demonstrate the presence of organisms not showing up in the usual plate culture technique but these were invariably unsuccessful.

The relation between bacterial numbers and abnormal reactions in the foremilk was found to vary from animal to animal, from quarter to quarter,

and even from time to time for the same quarter. In general, common udder micrococci were present in abnormally large numbers, but occasionally high count levels accompanied consistently normal values, and low count levels occurred in conjunction with definitely abnormal values. Some evidence was obtained that the onset of abnormalities in the composition of the secretion is preceded by a sharp rise in the level of counts. Changes in the secreting tissue resulting from previous bacterial invasion may explain the occurrence of abnormal reactions at a later date when the bacterial content is not unusually high; such phenomena have been observed in quarters definitely infected with pathogenic organisms.

In one animal (No. 12 U.W.) selected for intensive study, extraordinarily high counts (occasionally in excess of 500,000 per ml.) were noted quite frequently on the foremilk along with little evidence of abnormality in the biochemical reactions. However, examination of the strippings revealed some amazingly high values for catalase; those for pH were frequently much higher than those from the foremilk, while the chlorides showed only slight differences. (Table 1.) Subsequent examinations of the foremilk from this animal showed a definite trend toward higher biochemical values as the lactation period progressed, until none of the four quarters could be regarded as normal. It would indeed be surprising if such enormous numbers of bacteria could be

TABLE 1.—DATA ON SAMPLES OF FOREMILK AND STRIPPINGS.
HEIFER No. 12 (U.W.) APRIL, 1937

—	Left front	Right front	Left hind	Right hind
Bacterial Counts—				
13th a.m. F	3,700	2,520	145,000	4,500
S	630	9,200	30	640
13th p.m. F	460	1,680	1,700	2,600
S	21,100	900,000	1,720	90
14th a.m. F	5,500	160,000	160,000	56,400
S	9,800	5,200	910	6,000
Catalase %—				
13th a.m. F	11	6	27	5
S	178	122	184	81
13th p.m. F	15	14	26	13
S	157	.....	251	55
14th a.m. F	10	9	21	10
S	.....	133	79	59
pH—				
13th a.m. F	6·46	6·52	6·53	6·50
S	6·79	6·61	6·68	6·68
13th p.m. F	6·55	6·60	6·60	6·57
S	6·70	.....	6·86	6·62
14th a.m. F	6·56	6·56	6·56	6·56
S	6·93	6·76	6·67	6·67
Chlorides %—				
13th a.m. F	0·101	.....	0·100	.....
S	0·120	.....	0·095	1·112
13th p.m. F	0·100	0·105	0·105	0·097
S	0·119	.....	.....	0·100
14th a.m. F	.....	0·125	0·115	0·105

F—Foremilk.
S—Strippings.

present in the gland for considerable periods without appreciably affecting the composition of the secretion. Of course, it must be recognized that various factors other than bacterial infection may be responsible for changes in the composition of the secretion. The possibility that another biological agent, in addition to the bacteria, is concerned, warranted consideration. It seems not improbable that where the bacteria alone are present, the composition of the secretion is scarcely influenced, but when the hypothetical second agent is also present, definite changes occur. Although these studies have cast no light upon the possible nature of this second agent, its presence would serve to explain many of the inconsistencies noted in the foregoing paragraphs.

The significance of these abnormalities in secretion in the milk of animals free from infection with any recognized pathogen is difficult to estimate. Available evidence suggests that such cases are far more common than has hitherto been suspected. There is no evidence that this condition is contagious, and there seems to be no valid reason for demanding the removal of such animals from milking herds. In most instances the abnormalities in composition of the milk are confined to the foremilk or strippings, samples representative of the entire quantity drawn from the udder at a milking generally being well below the limits tentatively adopted for normal milk. There is no evidence of inflammation as indicated by the appearance of the udder or of the milk itself, and there appears to be little justification for attempts to exclude such milk from the fluid milk market.[1]. The effect upon milk yield is variable, some quarters showing serious reduction while others are only slightly affected. Final judgment as to the true significance of this type of secretion disturbance must await further information.[2]

THE CONTROL OF CHRONIC CONTAGIOUS MASTITIS

The control program inaugurated in November, 1935, at the Central Experimental Farm in co-operation with the Division of Animal Husbandry is based upon the cultural demonstration of infection with *Str. agalactiae.* The information gained by periodical examinations of the foremilk is used to classify the animals into three groups (*a*) clean, (*b*) suspicious, and (*c*) infected. They are stanchioned and milked in this order. Other measures adopted include a daily strip cup examination and recording of any abnormalities, and the dipping of the teats in a hypochlorite solution (400 p.p.m. Av. Cl.) after milking.

Despite these measures, five new cases of infection were detected during the twelve-month period. The streptococci were also demonstrated in one quarter of an udder from which they had been isolated once eighteen months before, but had failed to show up on twelve subsequent examinations. These additional infections probably resulted from the retention in the herd of several good breeding cows which were actively infected. Since these have been disposed of, no further new infections have been detected, while in the remaining infected cows the infection is rarely active as judged by the absence of strip cup reactions or visible inflammation.

For routine analysis, composite samples of all four quarters are taken and catalase and Hotis tests made at once. (The latter test also indicates whether the reaction is unduly alkaline, thus avoiding the necessity of a separate test for this purpose.) The remainder of the sample is held overnight at 5±° C. Next morning, Burri slants of sucrose-tryptone agar or Bowers and Hucker agar are inoculated from the cream layer, using a 3 mm. loop. Colonies resembling streptococci are fished to aesculin broth; those failing to hydrolyse aesculin are then seeded into litmus milk, methylene blue milk (1:5,000) and

[1] Studies are under way to determine the suitability of such milk for the manufacture of Cheddar cheese.

[2] For more detailed presentation of the findings on certain phases of this investigation, see Johns, C. K., and Hastings, E. G. Can. J. Research D, 16: 6-14, 15-30. 1938.

triple sugar broth (mannitol, inulin, raffinose) for identification. Where catalase values in excess of 30 per cent are encountered, individual quarter samples are taken at the first opportunity and subjected to a similar analysis. Where a typical reaction is noted with the Hotis test, the isolation of the organism from the tube is attempted.

Although some workers have reported unfavourably upon the Hotis test, it has given good satisfaction in this laboratory. On account of the much larger quantity of milk examined, it is frequently possible to detect a slight infection which escapes notice where the usual small quantity of milk is cultured on agar. The indication of pH furnished at the start has already been mentioned. Microscopic examination after overnight incubation may also be utilized where desired. The incubation of the tubes at an angle of 45° from the perpendicular has some advantage in facilitating the detection of the typical flakes or balls of yellow curd resulting from the growth of long chains of the streptococcus.

The possibility of modifying the Hotis test in such a manner that it may be applied to can or weigh-vat samples has received some attention. The addition of Brilliant Green in a concentration of 1:100,000 has been found to have some value in keeping down the growth of saprophytic contaminants, but further studies are necessary before its value can be definitely assessed.

FACTORS INFLUENCING THE RATE OF REDUCTION OF METHYLENE BLUE IN MILK

It has recently been proposed that changes be made in the salt of methylene blue, and in the dye concentration, employed in the methylene blue reduction test. Furthermore, in England a modified test, in which samples are first held for approximately 12-16 hours at outdoor temperature, and then inverted at half-hourly intervals during incubation at 37° C., has been adopted as the official method for the analysis of graded raw milks[1]. This appeared to be a favourable time for a more intensive study of a number of factors influencing the rate of reduction of methylene blue in milk. Investigations were therefore commenced in which it was planned to study the influence of the following factors:—

(*a*) Temperature of incubation
(*b*) Dye concentration
(*c*) Frequency of mixing contents of tubes
(*d*) Preliminary incubation at a lower temperature
(*e*) Preliminary storage at 5° C.

These studies will be described in detail in the report for the following year.

DAIRY CONTROL WORK

In addition to the investigational work reported above, the Division maintained a bacteriological control of the dairy operations at the Central Experimental Farm. Regular analyses were conducted on the raw milk, the pasteurized milk from the vat, and the same milk after bottling. In addition, surprise samples were taken from time to time to control methods employed by the milkers. The monthly average bacterial counts for the mixed raw milk from January, 1937, to March, 1938, are indicated in Table 2.

[1] Ministry of Health (England) Memo 139/Foods. January, 1937.

TABLE 2.—AVERAGE BACTERIAL COUNTS—MIXED RAW MILK

Month	1937	1938
January	4,020	2,780
February	4,390	6,320
March	4,180	7,000
April	8,380	
May	9,040	
June	11,610	
July	6,240	
August	5,040	
September	5,300	
October	2,630	
November	5,500	
December	4,030	

SOIL MICROBIOLOGY

LUGUME CULTURE DISTRIBUTION

From the establishment of the Division in 1923 until 1936, a service was maintained whereby cultures for the inoculation of legume seed were distributed free from this laboratory. The principal aim in this work was to encourage the practice of seed inoculation by sending any farmer on request sufficient material to treat one bushel of seed and thus make a trial on a practical scale. During the period 54,529 cultures were prepared and distributed by the Division. It is felt that this effort, carried out with the co-operation of the Dominion Experimental Farms throughout the country, has aided materially in making the farmers better aware of the advantages of seed inoculation and the part it plays in farming practice.

That the practice of legume inoculation is now better known is evidenced by the fact that whereas ten or fifteen years ago nitro-cultures were produced only at two or three governmental or institutional laboratories, they are now manufactured by several commercial concerns as well. In view of this development and the lessened need for culture distribution as a purely educational undertaking, the general distribution of nitro-cultures by the Division was discontinued in 1937. It was felt that with adequate sources of supply outside, the attention of the Division could be better directed towards investigational work on certain phases of the problem of legume inoculation still requiring study, such as the development of better strains of bacteria, with special consideration of their nitrogen-fixing power and ability to act in more acid soils.

Preparation and distribution of cultures is still maintained for co-operative work with Experimental Farms, Illustrations Stations and for special field tests. In 1937, 564 cultures were prepared for such purposes, chiefly for alfalfa, lupines, sweet clover and soybeans.

QUALITATIVE STUDIES OF SOIL MICRO-ORGANISMS

In September, 1936, a program of research was initiated dealing with fundamental aspects of soil microbiology, with special attention to the qualitative nature of soil micro-organisms[1]. Soil microbiological research has been directed for the most part towards a study of processes in which micro-organisms are known to participate rather than towards an objective study of soil micro-organisms themselves. Relatively little attention has been paid to groups of bacteria in soil whose functions are still unknown or but little understood, but which are believed to comprise a large proportion of the micro-population of

[1] Lochhead, A. G., and Taylor, C. B. Can. J. Research C, 16: 152-161. 1938.

arable soils. It is felt that studies of the nature and activities of such organisms should aid, not only in adding to the knowledge of soil and the part played by micro-organisms in soil fertility, but also provide basic information for attack on such practical problems as the relationship of normal soil micro-organisms to soil-borne diseases.

A SURVEY OF THE BACTERIAL FLORA OF SOILS DIFFERING IN FERTILITY

The object of the investigation (described in detail elsewhere[1]) was to study, on a non-selective basis, the bacterial types occurring in three soils and their relative incidence. The soils were of similar type and crop history, but by reason of different fertilizer treatment had become dissimilar in productivity. A previous study[2] of the same soils had been made with respect to their capacity for supporting nitrogen-fixing organisms, *Azotobacter* and species of *Rhizobium*. The soils were from a rotation of mangels, oats, clover and timothy, and for 25 years had been receiving respectively, no fertilizer, farmyard manure and artificial fertilizers. They contained approximately 0·11 per cent, 0·16 per cent, and 0·13 per cent nitrogen respectively and judged by crop yields represented soils with striking differences in crop producing ability.

Methods.—Samples were taken in September, 1936, for preliminary tests, and in November, 1936, February, April, and July, 1937, from plots which had produced a crop of timothy and which at the time of the July sampling were supporting a mangel crop. In September and November, samples were likewise taken from corresponding plots after a crop of mangels. Plate cultures were prepared and total cell counts made by the ratio method. Cultural studies of the general microflora require a medium as non-selective as possible. For this reason, soil extract agar without added energy material was chosen in preference to other media which, though synthetic and hence more easily reproducible, are regarded as more selective on account of the special energy sources contained.

The value of any qualitative study is enhanced when quantitative aspects are also taken into account. Haphazard selection or assumption of similar identity from observation of plate colonies are unsuited to an estimation of the relative incidence of different types. Consequently all colonies on representative plates or on a definite sector were transferred to soil extract semi-solid containing 0·02 per cent K_2HPO_4 and 0·01 per cent yeast extract. The use of this medium assured the survival of 92 per cent of all strains isolated. For group classification all cultures were examined morphologically and tested for physiological properties with respect to proteolytic action, utilization of sugar, and capacity for nitrate reduction.

Plate and Total Cell Counts.—A comparison of the soils from the point of view of "total numbers" is made in Table 3. The usual seasonal fluctuation is noted. The untreated soil appears less subject to fluctuation in numbers than the treated areas, particularly in cell counts. It is of interest to note that, although the plate count indicated that numbers were well maintained in frozen (February) soil, cell counts showed in all three cases a drop from the November sampling. The results fail to show any relation between numbers and crop yield. In the case of the soils sampled after mangels particularly, there was little variation in numbers of organisms between the unfertilized and the fertilized areas, in spite of very large differences in crop-producing ability.

[1] Taylor, C. B., and Lochhead, A. G. Can. J. Research C, 16: 162-173. 1938.
[2] Lochhead, A. G., and Thexton, R. H. Can. J. Research C, 14: 166-177. 1936.

TABLE 3.—TOTAL CELL AND PLATE COUNTS

(millions per gram dry soil)

	Following timothy			Following mangels		
	N	X	Y	N	X	Y
Yield (tons per acre) average 25 years	2·01	3·10	2·66	7·99	22·72	20·91
Yield in 1936	1·65	2·95	2·51	2·59	29·03	25·12
Total cell count—						
September	741·2	990·1	2,065·9	2,403·6	2,380·7	2,332·6
November	1,144·5	1,846·8	2,524·9	2,241·4	2,534·5	2,166·0
February	982·3	1,524·9	1,018·5	.....	.....	.....
April	681·3	1,071·5	925·3	.....	.....	.....
Plate counts—						
September	57·0	52·2	50·8	76·6	109·2	107·9
November	92·6	95·4	116·8	123·1	139·1	126·4
February	90·0	111·3	132·7	.....	.....	.....
April	72·4	81·1	60·0	.....	.....	.....
July	36·3	60·1	70·6	.....	.....	.....

Main Morphological Groups.—The organisms were divided into eight main groups on the basis of their morphology in semi-solid soil extract:—

Group I.—Short rods, Gram-positive
Group II.—Short rods, Gram-negative
Group III.—Short rods, Gram-variable
Group IV.—Short rods, changing to cocci (*Bact. globiforme* group)
Group V.—Coccoid rods, Gram-positive
Group VI.—Cocci, Gram-positive or negative
Group VII.—Long rods, non-spore-forming
Group VIII.—Spore-forming rods

The percentage distribution of the various groups in the soils following timothy is shown in Table 4. The physiological characteristics of the groups are summarized separately for the three soils and given in Table 5.

TABLE 4.—MAIN MORPHOLOGICAL GROUPS AT DIFFERENT SEASONS

(Soils following timothy)

	November			February			April			July		
	N	X	Y	N	X	Y	N	X	Y	N	X	Y
Soil moisture, %	15·7	17·2	18·1	24·1	32·3	26·2	24·9	23·1	26·1	13·5	15·9	17·6
Total cultures isolated	59	61	61	62	62	63	64	64	64	41	43	41
Percentage showing no growth on transfer	8·5	13·1	13·1	6·5	3·2	6·4	6·3	3·1	17·2	0·0	11·6	4·9
Percentage showing growth on transfer	91·5	86·9	86·9	93·5	96·8	93·6	93·7	96·9	82·8	100·0	88·4	95·1
Morphological groups												
Short rods, Gram-positive	24·0	28·3	35·8	39·6	46·6	30·5	28·3	17·7	9·4	24·4	28·9	20·5
Short rods, Gram-negative	37·0	26·4	32·0	25·8	15·0	35·6	26·6	53·2	56·6	34·1	50·0	46·1
Short rods, Gram-variable	18·5	18·8	9·4	3·4	10·0	13·5	3·3	11·3	15·0	2·4	0·0	0·0
Short rods, changing to cocci (*Bact. globiforme* group)	12·9	9·4	11·3	8·6	10·0	6·7	10·0	8·0	7·5	14·6	0·0	7·7
Cocci, Gram-positive or Gram-negative	3·7	3·7	5·6	10·3	3·3	1·7	6·6	4·8	1·9	0·0	0·0	0·0
Long rods, non-spore-forming	3·7	9·4	5·6	3·4	3·3	5·0	5·0	0·0	1·9	0·0	2·6	2·5
Rods, spore-forming	0·0	3·7	0·0	0·0	0·0	0·0	1·6	0·0	1·9	19·4	13·1	20·5

TABLE 5.—CHARACTERISTICS OF BACTERIAL GROUPS IN THREE SOILS

Groups	Per cent of total	Gr. on N. A. v. sl. or abs. %	Gelatin liquef. %	NO_3 reduction %	Soil-extr. s.s. + 1% dextrose		No action, gelatin, NO_3 or dextrose %
					No gr. %	Acid %	
Soil N (No fertilizer)—							
Short rods, Gram-positive	29·6	54·0	25·4	49·2	3·0	53·9	11·1
Short rods, Gram-negative	30·5	50·8	13·8	15·3	13·8	24·6	35·4
Short rods, Gram-variable	7·0	100·0	0·0	86·6	0·0	20·0	13·4
Short rods, changing to cocci (*Bact. globiforme* group)	11·2	0·0	100·0	33·3	0·0	75·0	0·0
Coccoid rods, Gram-positive	8·4	88·8	16·6	61·1	11·1	38·8	16·6
Cocci, Gram-positive or negative	5·6	91·6	33·3	66·6	8·4	41·6	0·0
Long rods, non-spore-forming	3·3	57·1	14·3	14·3	14·3	28·5	42·9
Rods, spore-forming	4·2	11·1	44·4	44·4	11·1	66·6	22·3
Soil X (Manure)—							
Short rods, Gram-positive	30·5	72·3	16·9	60·0	16·9	46·1	16·9
Short rods, Gram-negative	35·2	49·4	17·3	12·0	17·3	25·3	38·5
Short rods, Gram-variable	10·8	100·0	17·4	82·5	4·3	34·8	8·7
Short rods, changing to cocci (*Bact. globiforme* group)	7·5	0·0	100·0	25·0	0·0	75·0	0·0
Coccoid rods, Gram-positive	5·6	83·3	33·3	75·0	8·3	50·0	8·3
Cocci, Gram-positive or negative	3·3	100·0	14·3	85·7	0·0	42·8	0·0
Long rods, non-spore-forming	3·8	12·5	50·0	12·5	12·5	12·5	25·0
Rods, spore-forming	3·2	0·0	85·7	85·7	0·0	42·8	0·0
Soil Y (Artificial fertilizer)—							
Short rods, Gram-positive	24·5	84·0	18·0	48·0	14·0	46·0	30·0
Short rods, Gram-negative	42·1	64·0	19·7	5·0	23·2	37·0	38·3
Short rods, Gram-variable	10·3	100·0	33·3	66·6	4·8	42·8	4·8
Short rods, changing to cocci (*Bact. globiforme* groups)	8·3	0·0	100·0	52·9	0·0	82·3	0·0
Coccoid rods, Gram-positive	3·9	100·0	25·0	87·5	0·0	50·0	0·0
Cocci, Gram-positive or negative	2·4	80·0	20·0	20·0	20·0	0 0	60·0
Long rods, non-spore-forming	4·4	33·4	11·1	11·1	22·2	33·3	22·2
Rods, spore-forming	3·9	0·0	75·0	25·0	12·5	50·0	12·5

Non-sporing short rods were found to comprise a large proportion of organisms capable of being isolated from soils. The five groups into which short rods were classified made up 86·7 per cent, 89·6 per cent and 89·1 per cent respectively of cultures isolated from Soils N, X and Y, a surprisingly close agreement in soils differing so widely in productivity. Gram-negative short rods were found to be the most prevalent single groups of organisms, being rather more numerous than Gram-positive short rods in each soil, taken over the course of the four sampling periods. The difference was less pronounced in Soil N than in the fertilized soils X and Y. Variation in relative numbers was noted at different seasons. This group is seen to be the least active physiologically, and is suppressed to the greatest extent by the addition of dextrose, 42 of 226 strains being entirely inhibited by 1 per cent concentration.

Gram-positive short rods were the second most abundant group in all soils. This group displayed rather more activity than Gram-negative forms as judged by ability to reduce nitrates, liquefy gelatin or utilize dextrose. Like the latter group, however, a considerable percentage showed no growth on ordinary gelatin or agar and are believed to represent largely forms indigenous to soil only, brought out by such media as soil extract. Gram-variable short rods appeared to comprise a definite group failing to give a uniform reaction to Gram staining. Though numerically less important than either of the above groups, they showed certain characteristics which presumably justified their being classified separately. None of 59 strains isolated was able to grow on nutrient agar while their most pronounced biochemical feature was their comparatively high nitrate-reducing ability.

Cocci-forming rods classified as the *Bacterium globiforme* group were an important group in all soils studied, comprising 11·2 per cent, 7·5 per cent and 8·3 per cent of the organisms isolated from Soils N, X and Y respectively.

Members of this group, though definite rod forms in young cultures, show a change to the coccal form with longer incubation. Variations in cell size and rate of change from rod to coccus are noted between different strains of this group. Physiological tests further emphasized differences which may be exhibited by apparently closely related strains. Thus in a separate experiment in which 50 cultures of *Bact. globiforme* were compared as to ability to utilize six sugars, hydrolyze starch and reduce nitrates, 40 actual variations in physiology were noted. As a group these organisms were the most active of those found, and were uniform in ability to liquefy gelatin and grow on standard media. Coccoid rods, representing Gram-positive short rods that could not be satisfactorily differentiated from cocci, produced 8·4 per cent, 5·6 per cent and 3·9 per cent of the organisms isolated from Soils N, X, and Y. Like the Gram-variable short rods, the great majority failed to grow on nutrient agar, and included a large proportion of nitrate-reducing forms.

Micrococci, long rods and spore-forming types represent less prominent soil bacteria, judging from their abundance in the soils studied. Cocci comprised 5·6, 3·3 and 2·4 per cent of the strains isolated from the three soils. They showed much variation in type and appeared to represent a variety of species each present in but small numbers. Non-spore-forming long rods comprised 3·3, 3·8 and 4·4 per cent of the total organisms isolated from the three soils, while spore-forming rods were found to the extent of 4·2, 3·2 and 3·9 per cent. Only in the case of the July sampling did the last-named group represent an appreciable percentage of the forms isolated.

Comparison of Soils.—In Table 4, giving the incidence of the different groups in the three soils at different times, no outstanding differences are seen between the unfertilized soil, N, and the fertilized soils, X and Y, in spite of great variation in crop-producing capacity. The uniformity is the more interesting since soil X received an application of farmyard manure three weeks previous to the November sampling. Slightly higher percentages of non-spore-forming long rods and spore-formers were noted in this soil but otherwise the group incidence approximated closely that of the others. It seems that if notable increases in spore-formers are to result from addition of organic materials, amounts in excess of normal field applications are needed. It was not possible to correlate fertility with the relative abundance of any of the other main groups into which the organisms were classified. The soils in question, originally the same, had become different in crop-producing ability by artificial means, and the results suggest that in a soil of given type the bacterial flora may be fairly resistant to change, even though productivity may vary as much as tenfold.

There is indication of rather more difference in group incidence due to cropping than between the soils themselves. Comparative data, following timothy and mangels respectively, are given in Table 6, in which the approximate

TABLE 6.—BACTERIAL GROUPS IN THREE SOILS FOLLOWING DIFFERENT CROPS

Morphological group	Millions per gram of dry soil					
	Following timothy			Following mangels		
	N	X	Y	N	X	Y
Short rods, Gram-pos.	20·0	22·3	34·6	28·7	55·7	44·1
Short rods, Gram-neg.	30·8	20·9	30·9	16·4	23·2	27·3
Short rods, Gram-var.	15·4	14·9	9·1	24·6	16·2	14·7
Short rods, changing to cocci (*Bact. globiforme* group)	10·7	7·4	9·1	18·4	13·9	14·7
Cocci, Gram-pos. and Gram-neg.	3·1	3·0	5·5	8·2	0·0	12·6
Long rods, non-spore-forming	3·1	7·4	5·5	8·2	6·9	2·1
Rods, spore-forming	0·0	3·0	0·0	4·2	0·0	0·0

numbers in millions per gram dry soil are shown. After mangels, increased counts of Gram-positive and Gram-variable short rods and *Bact. globiforme* were found, as compared with the numbers following timothy.

Observations.—Micro-organisms in the untreated soil might be expected to represent largely indigenous soil organisms, contrasted with forms which may become more active upon addition of large applications of readily decomposable substances. The findings give little indication that the indigenous bacteria were noticeably affected by the treatments. The results suggest that growing crops have more influence upon the qualitative nature of the soil organisms. It is well known that within the soil zone influenced by the plant (rhizosphere) numbers of soil organisms are greatly increased. It is therefore of the greatest importance to study the *qualitative* effect of the growing plant on the soil micro-organisms. This is believed to be essential as a basis for a better understanding of such problems as the relationship of the normal soil micro-organisms to soil-borne diseases. The methods developed during the present work are being adapted to qualitative studies of the microbiology of the rhizosphere of crop plants.

Detailed study of the organisms isolated in this work showed a surprising variability in types apparently closely related. Furthermore the degree of biochemical activity displayed by the predominant soil organisms was regarded as relatively low (Table 5). It is suggested that the indigenous bacterial flora is comparatively unstable physiologically, and possessed of considerable adaptability. The comparative inactivity of so many forms when isolated from soil and cultivated singly also suggests that the functions of these species are exercised most fully only when they are acting in association with the other micro-organisms, and that for a proper understanding of soil biological processes greater attention must be given to reactions between plant and microbe as well as those between micro-organisms themselves.

A STUDY OF *BACTERIUM GLOBIFORME* IN SOILS DIFFERING IN FERTILITY

It has long been the aim of soil biologists to measure soil deficiencies and estimate general fertility by microbiological methods. Some success has been achieved in this direction through such procedures as the *Azotobacter* plaque method of Winogradsky and Ziemiecka, and the *Cunninghamella* method of Mehlick, Fred and Truog for estimating available phosphorus, and the *Aspergillus* method of Niklas and associates, used particularly for potash deficiency. There still remains the narrower and more fundamental question of relating any one type of organism to soil fertility. The search for such an organism the incidence of which in a given soil would serve as an index of crop-producing capacity, is considered justified in view of the practical application which would follow the discovery of such an indicator organism.

That the abundance of definite types of bacteria may be related to the productivity of soil was suggested by work previously reported,[1] which showed that the incidence of *Rhizobium meliloti* was consistent with the crop yields of three different soils throughout a four-year crop rotation. An organism more easily isolated by plating methods, *Bacterium globiforme*, had been found by Conn to thrive better in certain fertile soils than in certain less productive soils examined. In extension of the investigations reported above, special attention was paid to the occurrence of this organism in the three soils of unequal productivity. A detailed report of the methods and findings is given elsewhere.[2]

[1] Prog. Report, Dominion Agr. Bacteriologist for 1934-36. Dom. of Canada, Dept. Agr. 1938.
[2] Taylor, C. B., and Lochhead, A. G. Can. J. Research, C. 15: 340-347. 1937.

The approximate numbers of *Bact. globiforme* per gram of oven-dry soil are shown in Table 7, together with the crop yields of the corresponding plots for the

TABLE 7.—INCIDENCE OF *BACT. GLOBIFORME* IN SOILS OF DIFFERENT FERTILIZER TREATMENT

Soil	—	Crop yield (tons per acre)		*Bact. globiforme*—millions per gram, and percentage of plate count							
Treatment	Previous crop	1936	Average 20 years	Sept. 25, 1936 (tap water gelatin)		Nov. 12, 1936 (soil extr. agar)		Jan. 12, 1937* (soil extr. agar)		Feb. 23, 1937† (soil extr. agar)	
				No.	%	No.	%	No.	%	No.	%
N, no fertilizer	Timothy	1·65	2·01	5·8	35·8	10·5	11·6	7·7	6·7	6·9	7·7
X, manure	Timothy	2·85	3·10	2·3	25·7	7·5	7·8	3·9	3·3	10·2	9·2
Y, artificial fertilizer	Timothy	2·51	2·66	4·4	32·5	9·1	7·7	9·2	7·7	8·1	6·1
N, no fertilizer	Mangels	2·59	7·99	.....	.....	18·0	14·6	.....	.....	.....	.....
X, manure	Mangels	29·03	22·72	.....	.....	14·4	10·3	.....	.....	.....	.....
Y, artificial fertilizer	Mangels	25·12	20·91	.....	.....	14·7	11·6	.....	.....	.....	.....

* Soil from Nov. 12, 1936, held at 0°F. in refrigerator.
† Soil direct from field, frozen.

previous year and the 25-year averages. From the data there is no indication of any relation between the incidence of *Bact. globiforme* and the productivity of the soils. Greater numbers were found to occur after mangels than after timothy, which suggests an influence of the crop on the abundance of the organism. The soils studied, though varying in productiveness due to different fertilizer treatment, were of similar type originally, and hence it is possible that the lower incidence of *Bact. globiforme* noted in certain poor soils may be confined to unproductive soils of definite type. Further experiments, to be reported later, deal with a survey of soils from widely scattered parts of Canada, representing varied types and different grades of fertility.

Morphology.—The extent to which it may occur in soil indicates that *Bact. globiforme* is a numerically important type of the indigenous soil microflora. Little is understood of its function in soil. More interest has centred round its morphology, particularly the metamorphosis undergone in culture in the change from a definite rod-form to a coccus. All of 110 strains isolated from the soils showed this characteristic change, 10 of which were studied in detail. It is apparent that the rate of change from rod to coccus varied considerably with the strain as shown in Table 8, based on morphological comparisons.

TABLE 8.—METAMORPHOSIS FROM ROD TO COCCUS FORM IN TEN STRAINS OF *BACT. GLOBIFORME*

Culture No.	Cocci present in culture					
	17 hr.		41 hr.	65 hr.	6 days	
	%	Mean cell length (μ)	%	%	%	Cell diam. (μ)
NM4	0	1·6	10	50	95	0·7–1·0
XM51	80	1·8	100	100	100	1·0
YT34	10	1·8	40	60	95	0·9–2·0
YF2	20	1·6	90	90	95	1·0
YG12	10	1·8	20	85	95	0·7–1·2
NG53	2	1·4	95	100	100	0·8–1·0
XG11	5	1·4	60	80	80	0·8–1·5
YT54	0	1·4	100	98	100	1·0
YG11	5	1·9	80	90	100	0·7
29	0	1·6	20	40	90	0·9–1·5

The metamorphosis of the rod to coccus is most interesting and a striking example of pleomorphism. Formerly considered as merely a shortening of the rod until the cells become spherical, the change would appear to be less simple. The rods, characteristic of young cultures, generally become granular and begin to distend, usually at one extremity. The swelling continues until the rods are club-shaped and bent, after which the remainder of the rod breaks away leaving an almost spherical body with a faint tail. Later the tail disappears and the cell has the appearance of a coccus.

FOOD BACTERIOLOGY

A STUDY OF MICROCOCCI SURVIVING IN FROZEN PACK VEGETABLES

In the course of investigations previously reported,[1] dealing with the survival of micro-organisms in frozen pack vegetables, great variability was noted in resistance to prolonged freezing displayed by different groups of bacteria. Micrococci (including staphylococci) were found to survive better than other groups so that they comprised a much larger percentage of the organisms in the frozen, as compared with the fresh pack. Owing to attention which has been focused on this group of bacteria in connection with certain food-poisoning outbreaks, involving gastro-enteric disturbances following ingestion of foods (in most cases of starchy nature), investigations were undertaken to study frozen vegetables in relation to staphylococcal food poisoning from two points of view:

(1) To note the occurrence of staphylococci of food-poisoning type in frozen packs.
(2) To study the capacity of food-poisoning types to grow and produce enterotoxic substance under various conditions of handling the frozen and defrosted product.

Of 3,226 colonies of bacteria isolated from frozen packs from 1934 to 1936, including asparagus, spinach, peas, beans, and corn, a total of 1,490 proved to be micrococci. Of these, 980 cultures, from the 1935 and 1936 packs, were used in these present experiments. To facilitate experimentation it was necessary to eliminate replicates and comparative studies of the strains reduced the number to 50 different types. In addition, tests were made with 4 strains from the late Dr. E. O. Jordan, Chicago, and 5 from Dr. C. E. Dolman, Toronto. All strains from Dr. Jordan had been implicated in food poisoning outbreaks while those from Dr. Dolman were isolated from raw milk, or from food poisoning outbreaks, with one strain from nasal mucous membrane. All were considered enterotoxic strains according to the methods used by these investigators.

Detection of Enterotoxic Strains.—Details of methods used and results obtained are available elsewhere.[2] The procedure for the detection of enterotoxic substance consisted in cultivating the organism on semi-solid nutrient medium containing 1 per cent starch in Kolle flasks incubated at 37° C. in an atmosphere of 25 per cent carbon dioxide and 75 per cent air. After 48 hours' growth the culture was filtered through cheesecloth, then filter paper and finally through an "N" Berkefeld filter.

The filtrates were fed to kittens, 3 to 5 months old. Preliminary tests indicated that the intraperitoneal injection method, as recommended by Dolman, left much to be desired on account of disturbances resulting from the inoculation of uninoculated control filtrates. The procedure finally adopted was a new one, consisting in feeding 5 ml. of unheated filtrate by pipette. This method not

[1] Lochhead, A. G., and Jones, A. H. Food Research, 1: 29-39. 1936; 3: 299-306. 1938. Summarized in Prog. Rep't. Dominion Agr. Bacteriologist for 1934-1936.
[2] Jones, A. H., and Lochhead, A. G. Food Research, 4: in press. 1939.

only obviated dilution of the filtrate (by milk, etc.) but permitted the administration of a definite quantity. Typical symptoms were diarrhoea and vomiting, the latter at times slight. Diarrhoea was a regular symptom, positive reactions, generally occurring 2 to 5 hours after feeding toxic filtrates, usually 3 to 4 hours.[1]

Results of Feeding Tests.—Of the 50 strains of micrococci from frozen pack vegetables representing 980 isolations, 12 strains were positive. All of the vegetable products were shown to harbour one or more strains capable of elaborating enterotoxic substance when grown in a semi-solid medium in an atmosphere of CO_2 and air and tested by the pipette feeding method.

Correlation of Feeding Reactions with Biochemical Tests.—A correlation of the results of the kitten feeding reactions with biochemical tests of the organisms is summarized in Table 9, bringing out some points of interest.

TABLE 9.—BIOCHEMICAL REACTIONS OF MICROCOCCI

—	Feeding reaction positive	Feeding reaction negative
Cultures tested (58)	20	38
	per cent	per cent
Acid from lactose	100·0	44·7
Acid from mannite	100·0	47·4
Acid from maltose	90·0	65·8
Acid from sucrose	95·0	71·1
Acid in milk	90·0	36·8
Milk curdled	70·0	26·3
Nitrate reduced to nitrite	90·0	68·4
Hemolysis	60·0	23·7
Liquefaction, nutrient gelatin	70·0	57·9
Liquefaction, Stone's gelatin	65·0	13·2

Cultures showing positive reactions on feeding were more active physiologically. All cultures positive for kittens fermented lactose and mannite, suggesting preliminary tests with these carbohydrates as a means of eliminating many non-toxic strains. Of further interest is the fact that Stone's gelatin medium, proposed for the detection of food poisoning staphylococci, did not provide a means of differentiating between cultures.

Suitability of Vegetable Products for Growth of Food-poisoning Staphylococci.—Capacity for growth of food-poisoning stayphylococci in vegetable products held at different temperatures was studied with peas and asparagus. Using strains isolated from food poisoning outbreaks similar lots of vegetables, after inoculation, were held at room temperature and in a refrigerator at approximately 40° F., respectively, half of the samples having been previously frozen for one week. Quantitative determinations indicated that in both frozen and unfrozen lots, staphylococci of food-poisoning type may develop rapidly provided the products are held at room temperature. On the other hand, at the temperature of the refrigerator (40° F.) growth is definitely inhibited.

Production of Enterotoxic Substance in Frozen Pack Vegetables.—Since it was found that certain strains of micrococci isolated from frozen vegetables would produce enterotoxic substance in a semi-solid starch medium in an atmosphere of 25 per cent CO_2, further study was made to note whether the same organisms would form this substance in a packed vegetable. After packing

[1] The Division is indebted to the Laboratory of Hygiene, Department of Pensions and National Health, for the provision of facilities to conduct the feeding tests.

and inoculating, samples of corn were frozen for 1 week at 0° F., after which different lots were held at different temperatures. Filtrates were obtained and used for kitten feeding tests. It was found (Table 10) that of 18 cultures tested,

TABLE 10.—PRODUCTION OF ENTEROTOXIC SUBSTANCE IN FROZEN PACK CORN

Packs held after inoculating and freezing	Number of cultures tested	Reaction with kittens	
		Positive	Negative
Room temperature, 1 day	18	8	10
Ice-box (40–50°F.), 2 weeks	18	0	18
Electric refrigerator (40°F.), 2 weeks	18	0	18

8 strains gave positive reactions, but only in the case of corn held at room temperature. None of the samples defrosted and held either in the ice-box or in the electric refrigerator gave filtrates showing any toxic effect. It was found, however, that enterotoxic substances, once they are formed (e.g. by storing at higher temperature), are not impaired by holding at 40° F. up to two months.

Practical Considerations from Frozen Pack Studies.—The microbiological investigations on the packing and handling of frozen vegetables and fruits, which have extended over a period of four years, have brought to light practical considerations of importance for the industry. It has been shown that although marked decreases in numbers of micro-organisms—bacteria, moulds, yeasts—may occur after freezing, the process is by no means one of sterilization. Even after months of storage at 0° F. frozen products contain sufficient living organisms to develop after thawing and cause spoilage and make the products hygienically unsound. Freezing exerts a selective action on the bacteria originally present, permitting micrococci to survive in relatively much greater numbers than other types.

Frozen vegetables and fruits may be kept safe under reasonable handling to prevent development of organisms causing spoilage. This involves, apart from care in washing and blanching to minimize the microbial load on the fresh pack, prompt and uninterrupted freezing and prompt consumption after defrosting. Moreover the studies indicate emphatically that products once defrosted should not be re-frozen. So far no case of food poisoning traceable to frozen vegetables or fruits has come to our attention. The observance of simple precautions should maintain frozen pack products as safe and wholesome foods.

MICROBIOLOGY OF BACON CURING

During the year continued attention was given to microbiological problems of bacon curing with special consideration of the Wiltshire process. In a previous report[1] preliminary work was summarized which dealt with methods for the examination of curing brine and with a qualitative study of bacterial types present in Wiltshire pickle. The latter work indicated pronounced differences in the types of bacteria found depending upon the salt concentration of the medium used for their isolation. Whereas micrococci predominated on media without salt, rod forms, largely halophilic, formed the greatest number of organisms detectable on media of high salt content (15 per cent). The work showed the presence in pickle, not only of salt tolerant bacteria, but also of organisms able to resist, if not multiply in, the high salt concentrations found in curing brine.

[1] Progress Report of Dominion Agr. Bacteriologist for 1934-36.

Methods for Estimating Bacteria in Curing Pickle.—Further data were obtained on methods for estimating numbers of bacteria in curing pickle by the plating method. The importance of the temperature of incubation and the salt content of the medium was clearly brought out. In Fig. 3, showing the relative counts from the analysis of 50 samples of pickle, it is seen that maximum numbers were obtained with a medium containing 10 per cent salt.

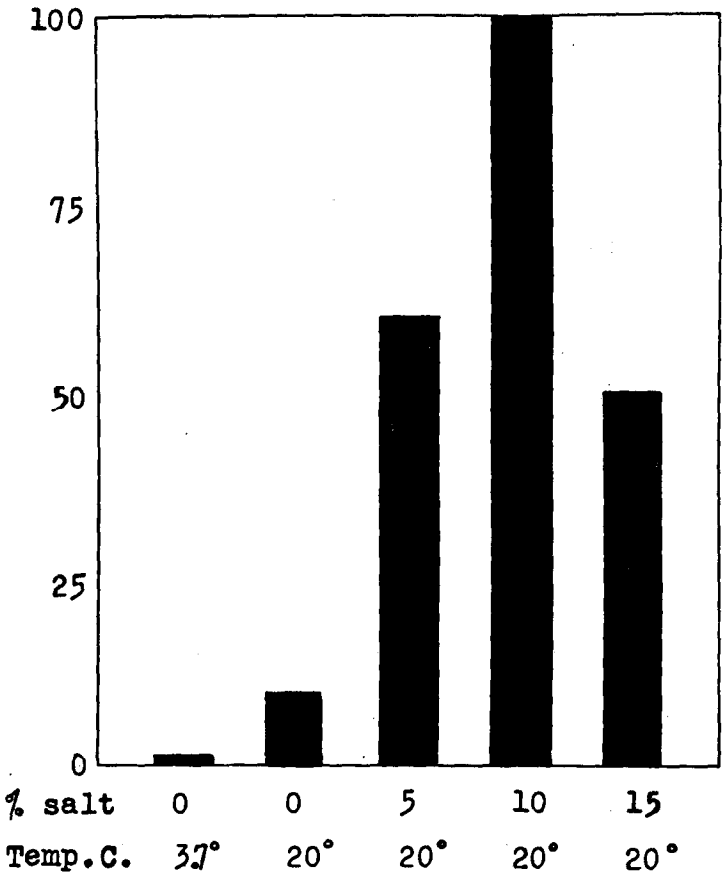

FIG. 3.—Effect of salt content of medium and temperature of incubation on bacteria counts of curing pickle

In plating on 10 per cent salt agar it is of the utmost importance to use dilution blanks of suitable salt concentration. This is indicated plainly in Fig. 4, based on averages from 7 samples plated on different media with dilution blanks containing 10 per cent salt and tap water respectively. Exposure to tap water kills or renders incapable of growth the great majority of organisms from pickle which are capable of growth on 10 per cent salt agar. As expected, numbers of bacteria from such an environment capable of growth on nutrient agar without salt, are unaffected by the salt content of the dilution blank.

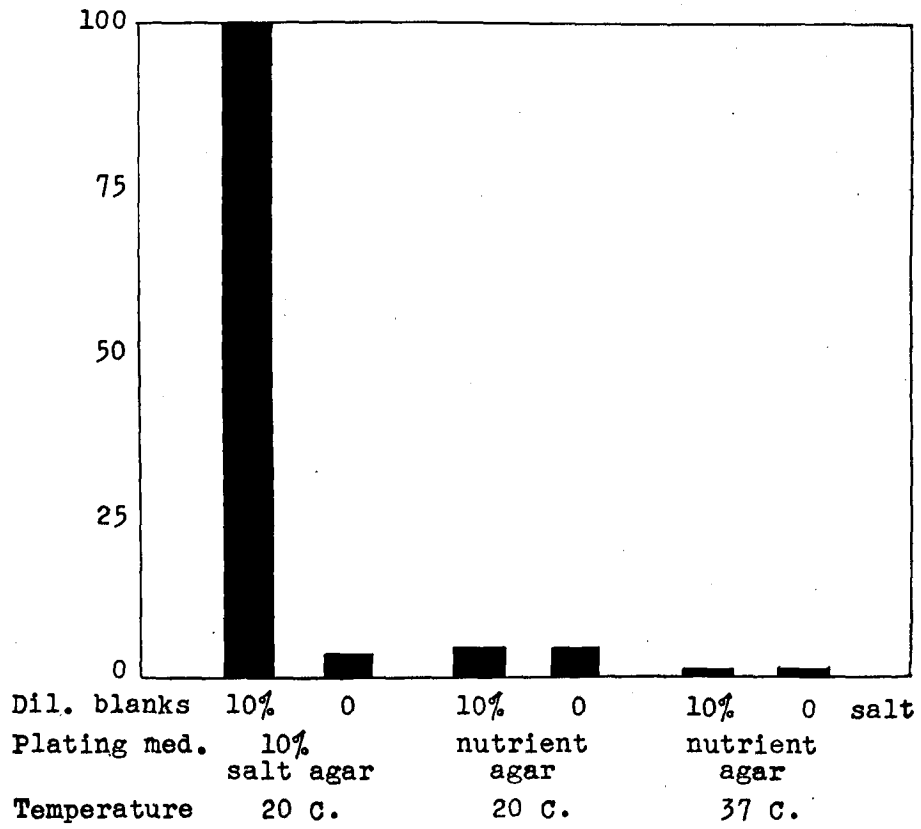

FIG. 4.—Effect of salt content of dilution blanks on bacteria counts of curing pickle.

Observations of Wiltshire Sides at Different Stages.—Attempts were made to study surface contamination of Wiltshire sides at different stages from a qualitative standpoint, with consideration also of the incidence of organisms of different salt requirements. Numbers were estimated by a filter paper impression method developed by Landerkin in this laboratory.

A trend of the surface load is indicated in Fig. 5. At killing contamination was low, with relatively fewer salt-tolerant organisms. At cutting prior to pickling increased numbers are found. After pickling and draining, prior to wiping, the effect of the pickle in changing the relative incidence of different types is noted. Wiping effects a reduction, though after baling and storage increased numbers are noted, particularly of those growing on 5 per cent salt medium.

Organisms able to grow without salt, comprising the largest group prior to pickling, were found to persist, though in diminished numbers, after wiping. This group definitely increased during storage. The findings are suggestive when considered with those of the qualitative studies of pickle bacteria previously reported, which showed types able to withstand, if not grow in, high salt concentrations. The possibility is indicated that deleterious organisms contaminating sides prior to curing, may carry through and cause later defects. Direct studies on the importance of pre-curing contamination in relation to Wiltshire processing were commenced towards the end of the year under review and will be reported later.

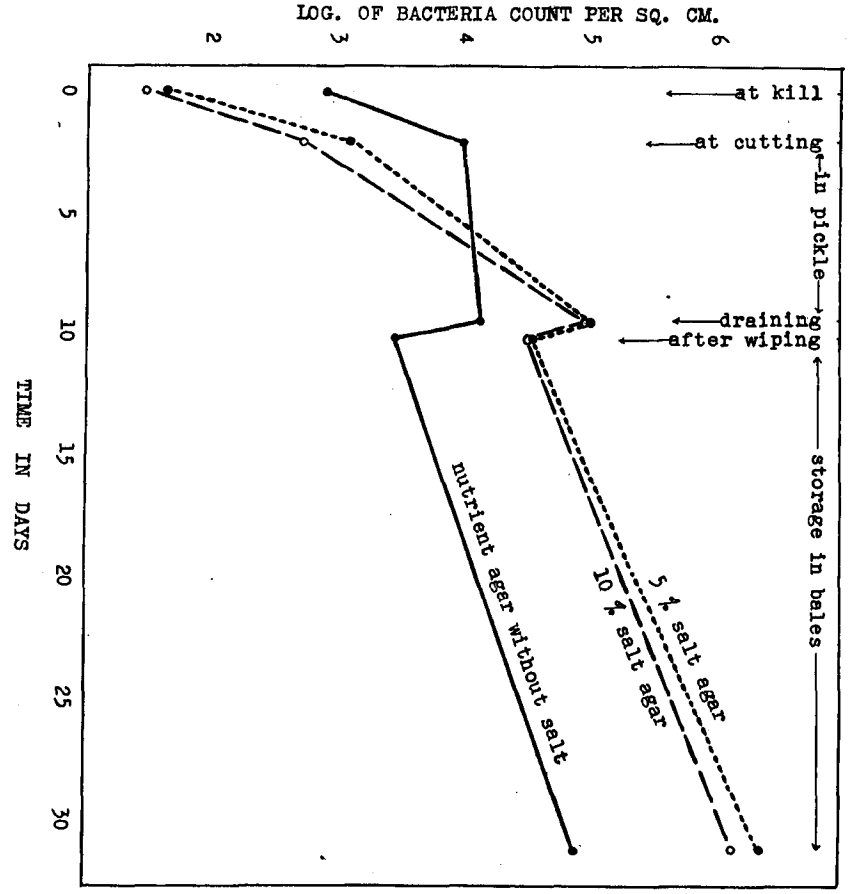

FIG. 5.—Illustrating "follow through" test of Wiltshire bacon packing. Relative surface bacterial load at different stages.

CANNED TOMATO PRODUCTS

In connection with the control of canned tomato products maintained by the Fruit Branch, the Division of Bacteriology has co-operated in studying the sanitary quality of tomato juice, pulp, puree, paste and catsup. Following extensive preliminary surveys in 1933 and 1934 standards based on the Howard Mould Count were adopted for the control of such products.

An indication of the improvement effected since the adoption of the standards in 1935 is given by the summaries in Table 11. Through better con-

TABLE 11.--TOMATO PRODUCTS—MICROANALYTICAL CONTROL

Year	Number of samples examined	Howard Mould Count[1] (Median Count)				
		Juice	Pulp	Puree	Paste	Catsup
1933	76	13	50	66	.....	.....
1934	221	8	32	50	.....	.....
1935	821	2	18	24	48	22
1936	900	4	24	20	38	14
1937	1,105	4	22	18	.....	10

[1] Percentage of microscopic fields showing mould when sample is examined according to the Howard technique.

trol and co-operation with canning plants a general lowering of counts has resulted.

Control work has also been maintained on imported products. During the year 1937 a total of 280 samples from import shipments, of which 264 were tomato paste, were analysed. Of these 230 or 82·1 per cent were within the limits, leaving 50 or 17·9 per cent in excess of the standard mould count permitted.

BACTERIOLOGICAL CONTROL OF EDIBLE GELATIN

In co-operation with the Meat and Canned Foods Division, Health of Animals Branch, this Division continued in the work of bacteriological control of gelatin for use in foodstuffs. Following upon the adoption of standards which came into force in 1936, careful supervision of edible gelatin, which is an import product, has been maintained by the Health of Animals Branch. The noticeable improvement which has been effected in the hygienic quality of edible gelatin since the inauguration of the control measures in 1936 may be seen from Table 12.

TABLE 12.—EDIBLE GELATIN—BACTERIOLOGICAL CONTROL

Year	Number of samples	Within limits,[2] total count and *B. coli*	Above limit, total count or coli
		per cent	per cent
1935	102	52·9	47·1
1936[1]	281	56·9	43·1
1937	1,191	82·7	17·3

[1] First year of enforcement.
[2] Total count, 10,000 bacteria per gram; *B. coli*, absent from 1/100 gram.

MISCELLANEOUS

OBSERVATIONS ON HYDROGEN SULPHIDE PRODUCING BACTERIA IN HARBOUR WATER

When iron or steel is subject to the action of hydrogen sulphide it may suffer considerable corrosion when moisture is present. Favourable conditions may exist for such corrosive action when the metal is buried in soil or immersed in water. Provided sulphates are present and anaerobic conditions prevail, hydrogen sulphide may be formed through the action of a very specialized group of sulphate-reducing bacteria. They are a group widely distributed in nature and their presence in soil, canal water, sea bottom slime, and water from oil wells has served to draw attention to their economic importance.

During the year observations were made on samples of water from an Atlantic harbour and furnished through the National Research Council. The presence of hydrogen sulphide noted at times in the water and its content of sulphate suggested an active flora of sulphate-reducing organisms.

In all samples the presence of anaerobic sulphate-reducing vibrio forms was noted. These organisms were noted to live in close association with aerobic forms, the presence of which, through oxygen consumption, makes for better growth of the former. Semi-solid medium, with sulphate as the sole source of sulphur, was found particularly useful in detecting sulphate-reducing forms. The formation of H_2S was favoured by the presence of salt within the ranges occurring in sea water. In Fig. 6 is illustrated the effect of salt

concentration on sulphate reduction by forms occurring in sea water. Presence of H_2S is indicated by the darkening due to reaction of the gas with ferrous ammonium sulphate added to the medium. Though in some cases production of H_2S was detected with concentrations as high as 3·5 per cent, the optimum salt content was in the neighbourhood of 1·5 per cent.

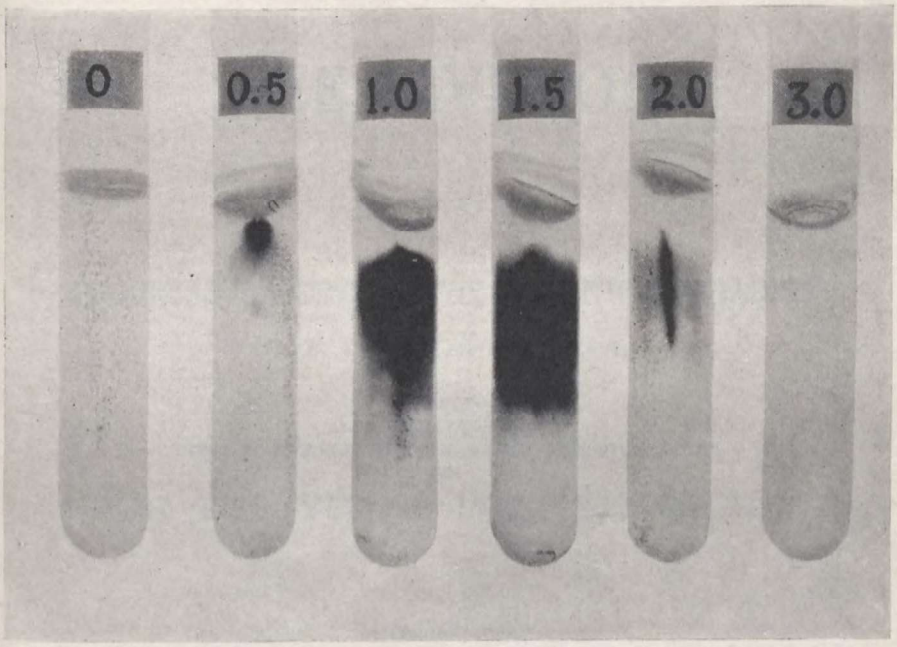

FIG. 6.—Relation of salt concentration to hydrogen sulphide production by sulphate-reducing bacteria from harbour water.

The activity of bacteria producing hydrogen sulphide by reduction of sulphates may be favoured by special conditions. It is encouraged by the presence of readily decomposable organic compounds able to serve as source of energy for the reduction process. This may result from abundance of organic material in the sea bottom or from addition of sewage, which latter also adds to the sulphate content of the water. Furthermore in bodies of water with less flow and change, H_2S formation is favoured. Thus in water further from shore in the same harbour, H_2S forming organisms were present to a very much smaller extent.

GENERAL ANALYTICAL SERVICE

In continuance of the policy maintained in previous years, assistance was given to the farming community, and to other Divisions and Branches of the Department, through the examination of samples submitted for bacteriological analysis. During the year 1937 a total of 2,850 samples was received, exclusive of routine milk samples from the Central Experimental Farm dairy, and a further total of 925 to March 31, 1938. The samples consisted of waters from farm wells, milk and other dairy products, honey, canned goods, meats and other foodstuffs, legume inoculants, foulbrood specimens, and others of an agricultural nature. From the diversity in character of the samples submitted the analytical work involved varied greatly. While some samples required but routine testing, others necessitated a much more extended investigation.

OTTAWA
J. O. PATENAUDE, I.S.O.
PRINTER TO THE KING'S MOST EXCELLENT MAJESTY
1939