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DEPARTMENT OF AGRICULTURE
DOMINION EXPERIMENTAL FARMS

DIVISION OF BACTERIOLOGY

PROGRESS REPORT OF THE DOMINION AGRICULTURAL BACTERIOLOGIST

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FOR THE YEARS 1934, 1935, AND 1936

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DOMINION EXPERIMENTAL FARMS

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DIVISION OF BACTERIOLOGY

Progress Report for the Years 1934-35-36

INTRODUCTION

The present report describes briefly the work and progress of the Division of Bacteriology during the three-year period 1934-36. The activities of the Division consist in: (*a*) research in microbiology as applied to milk production, soils and soil fertility, food and 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 of specimens, preparation of seed inoculants, etc.; (*c*) co-operation in administration of acts and other regulatory services under the Department of Agriculture.

During the years under review research has comprised the greater part of the work of the staff of the Division and this report is concerned mainly with progress in investigatory work in the fields indicated.

DAIRY BACTERIOLOGY*

Contamination from dairy utensils represents one of the most serious sources of germ contamination of milk as ordinarily produced on our farms. Consequently any general improvement in the quality of raw milk will depend upon the means taken to maintain pails, cans, strainers, etc., in proper sanitary condition. Though utensils may be adequately sterilized with steam or abundant hot water, effective treatment by these agents is not always practicable on many farms.

Since sterilization of dairy equipment by chlorine compounds offers a practical alternative to the use of steam or hot water, considerable attention has been given to a study of the action of chlorine compounds and factors affecting their germicidal efficiency. The findings are important not only to the dairying industry but to all industries dealing with foodstuffs, where sterility of equipment is important.

THE EVALUATION OF THE GERMICIDAL POTENCY OF CHLORINE COMPOUNDS. I. HYPOCHLORITES

With the increasing employment of chlorine sterilization in the dairy and other food industries, many new commercial chlorine products have appeared on the market. Previous studies[1] having shown that these products differed enormously in germicidal speed, prospective purchasers wish to know which are quick-acting and which are not. Extensive studies by this Division indicated that testing methods previously employed were open to serious objections and considerable time was spent in attempting to develop modifications of these tests. Finally a totally different method was devised which is believed to be much superior. In this, known as the glass slide method, the test organism is present in a film of diluted milk on the surface of a glass slide which is gently

*The section of this report dealing with Dairy Bacteriology has been prepared by C. K. Johns.
[1]Report of the Dom. Agric. Bacteriologist for the years 1929 and 1930. Dom. of Can. Department of Agriculture, 1931.

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agitated in the chlorine solution, thus approximating more closely the actual conditions under which chlorine solutions have to work in the sterilization of dairy utensils and equipment.[2]

The influence of added alkali in slowing down the germicidal speed of chlorine solutions has been noted by various workers, but none of the existing methods permitted the determination of the pH in such strongly oxidizing solutions. However, with the development of the glass electrode and vacuum tube potentiometer, it became possible to obtain accurate pH values for this type of solution. When this was done on a long series of products of varying alkalinity it was found that a perfect agreement existed between increase in pH and the slowing down of germicidal speed, regardless of the type of alkali employed to stabilize the solution. This agreement is well illustrated by the data on a series of eleven products, presented in table 1.

TABLE 1.—GERMICIDAL POTENCY OF NaOCl SOLUTIONS AT 100 P.P.M. AV. CL. AS MEASURED BY GLASS SLIDE TECHNIQUE AT 20°C.

Product	pH	Periods of Exposure (Seconds)																					
		1	5	10	15	20	30	40	50	60	75	90	120										
B-K	9·2	**14**	4	0	0	0	0																
Merclor	9·8	**1**	0	1	0	1	0																
HTH	10·1	**2**	1	0	0	1	0																
HTH-10 B	10·6				**9**		2	1	0	0	7	3	0	1	0								
Chlorosil	10·9					215		**53**	**10**	7	0	1	0	2	0	0							
HTH-15	11·4						+		+	+	412	290	**38**	**37**	0	0							
Antiformin	11·52						+		+		+	+	142	36	**1**	**4**	1		0				
Diversol (new)	11·56						+		+		+		+	+	**31**		6		0				
Pure-A-Fac	11·65						+		+		+	+	+	+	72	**9**	2		1				
Neomoscan	11·7								+		+		+	+	270	121	8	4	1	0			
Diversol (old)	11·95								+		+		+	+	+	+	41	18	0	0			

N.B.+=too numerous to count.
The counts in black type mark the shortest exposure period where the average value indicates 99·75% destruction.

The above findings suggested that the efficiency of a hypochlorite product might be evaluated by pH determination instead of by the more involved bacteriological method. After considerable experimentation a simple colorimetric method was devised by which a series of hypochlorite products could readily be ranked in the order of their germicidal efficiency.[1]

Studies of the influence of dilution upon germicidal potency led to some surprising findings. When a series of products was tested at 100 parts per million (p.p.m.) available chlorine and then tested again at 2 p.p.m. it was found that certain products were relative more (or less) effective at the lower concentration. This was found to be due to the relative buffer capacities of the solutions, products poorly buffered increasing in efficiency to a much more marked degree than those more strongly buffered. These findings emphasized the importance of carrying out tests with chlorine solutions at the actual concentration at which they are to be employed in practice.

[2]For details of studies on testing methods, see Johns, C. K., Sci. Agr. 14: 585-607. 1934.
[1]For details, see Johns, C. K. Sci. Agr. 14: 585–607. 1934.

Further studies indicated that, contrary to all expectations, the germicidal potency may actually increase upon dilution. The explanation of this anomalous behaviour lies in the fact that the concentration of hypochlorous acid (the germicidally active ingredient of chlorine solution) is a function of pH. (See figure 1.) If therefore the pH change on dilution is sufficiently marked, the more dilute solution may actually contain a greater concentration of hypochlorous acid than the undiluted solution.

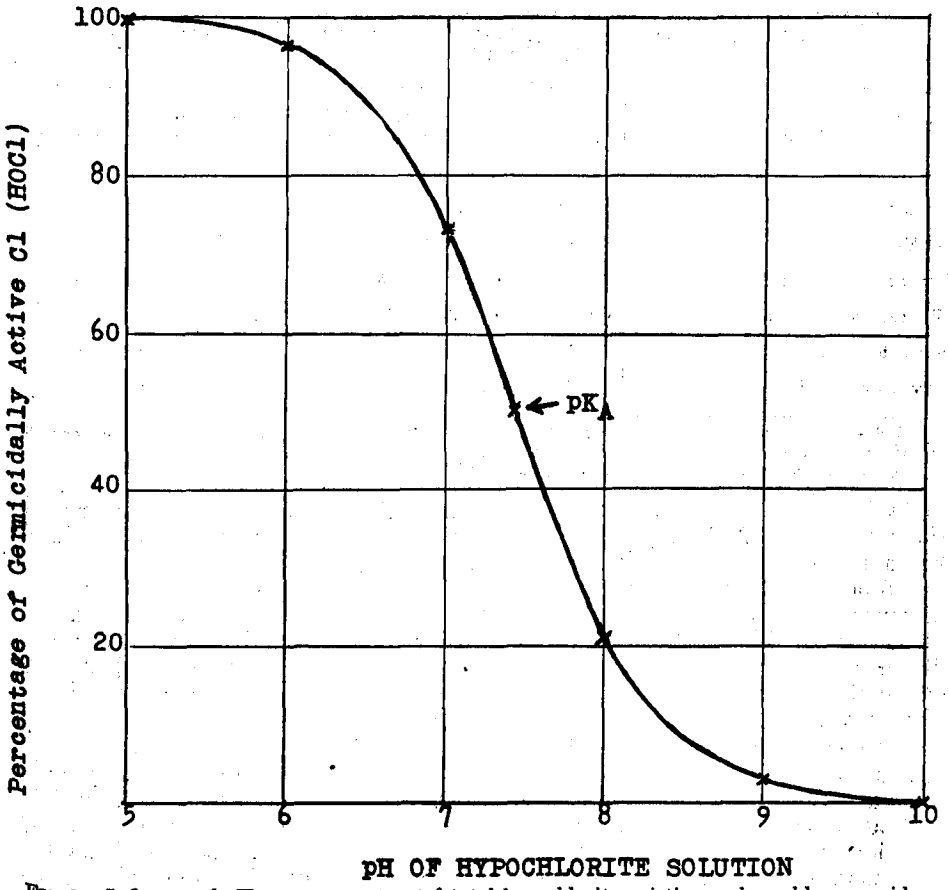

FIG. 1.—Influence of pH upon percentage of total hypochlorite existing as hypochlorous acid.

These findings suggested that under ordinary conditions of use there was an enormous wastage of chlorine. If the pH of the solution could be reduced, effective sterilization should be possible with much weaker chlorine concentrations. Plant studies proved this reasoning to be sound, excellent results being obtained with flush solutions of 1 or 2 p.p.m. acidified to pH 6·0.[1]

THE EVALUATION OF THE GERMICIDAL POTENCY OF CHLORINE COMPOUNDS. II. CHLORAMINE-T PRODUCTS

In the literature numerous contradictory reports appear concerning the relative efficiency of chloramine-T (sodium paratoluene-sulphonchloramide) and hypochlorite. Since no information was available comparing the C.P. product with the commercial sterilizing compounds, a series of studies was undertaken similar to those reported for the hypochlorites.

[1]See Johns, C. K., Proc. Internat. Assoc. Milk Dealers, Lab. Sec. 27: 49-64. 1934.

Since chloramine-T is much more stable than hypochlorite in the presence of excess organic matter, the majority of testing methods afforded erroneous impressions of their relative efficiency. However, by the use of the glass slide technique previously mentioned, these and other objections were avoided and satisfactory evaluations of relative potency were made.

As in the case of hypochlorites, the relative germicidal potency varies with pH. Data on chloramine-T, U.S.P. and five commercial products are presented

TABLE 2.—GERMICIDAL POTENCY OF SIX CHLORAMINE-T PRODUCTS (2000 P.P.M. AV. CL.) AS MEASURED BY THE GLASS SLIDE TECHNIQUE. 20°C.

Product	pH	Period of Exposure (minutes)								
		½		¾		1		2		5
Sterichlor	8·32	**1**		0	0	0	0	0		
XCEM	8·38	**0**		0	0	0	0	0		
Wyandotte Sterilizer	8·50	**70**		5	2	5	0	0		
Chloramine-T U.S.P.	9·18	**99**[1]	**31**[1]	189	65[1]	2[1]				
Klenocide (liquid)	9·20	–		–		–	28[1]	**1**	**1**	**0**
Klenocide (powder)	9·84	–		–	–	–	–	208	81	**0**

N.B.—Estimated count 35,000 on control plates. The counts in black type mark the shortest exposure period where the average value indicates 99·75% destruction.

[1]Spreaders on these plates probably resulted in unduly low counts.

in table 2 which illustrate this relationship. The concentration of chlorine in these tests was 2,000 p.p.m., indicating beyond any doubt that chloramine-T has much less germicidal potency than hypochlorite.

Studies with these six products at different dilutions led to the amazing discovery that with the five commercial products the pH was actually greater at 200 p.p.m. than at 2,000 p.p.m. The chloramine-T, U.S.P. on the other hand declined in pH on dilution in much the same manner as the hypochlorites. The explanation for this anomalous behaviour lies in the presence of sodium bicarbonate as a " filler " in the commercial products.

While the commercial products become steadily less effective as the concentration of chlorine is reduced, the chloramine-T, U.S.P. shows peculiar fluctuations on dilution. As the concentration of chlorine is reduced from 5,000 to 1,000 p.p.m. the germicidal efficiency declines, followed by a definite increase in efficiency to a point at approximately 200 p.p.m. This peculiar behaviour may also be explained on the basis of the effect of dilution upon pH if it is assumed that hypochlorous acid is the germicidally active constituent.[1]

TESTS ON METHYLENE BLUE THIOCYANATE FOR USE IN THE METHYLENE BLUE REDUCTION TEST

At the request of Dr. H. J. Conn, Chairman of the Commission on Standardization of Biological Stains, a series of tests was conducted comparing methylene blue thiocyanate with the standard tablets prepared from methylene blue chloride. The fact that the former can be prepared with a purity approximating 100 per cent while the latter is extremely difficult to standardize, has suggested that the thiocyanate might be substituted for the chloride. The Commission wished to ascertain therefore, (1) whether the thiocyanate could be substituted for the chloride with no change in results, and (2) whether some other concentration than 1 part in 700,000 of milk would be more desirable.

[1]For details concerning studies on chloramine-T products see Johns, C. K. Sci. Agr. 15: 218-227. 1934.

Samples of mixed night's and morning's milk were obtained for testing and replicate tubes prepared. Results with the two different lots of thiocyanate have been combined to furnish the summarized data presented in table 3. It

TABLE 3.—AVERAGE REDUCTION TIMES OF MILKS CONTAINING VARIOUS CONCENTRATIONS OF SALTS OF METHYLENE BLUE AT 37°C.

—	Thiocyanate[1]			Chloride (Standard tablets 1: 700,000)
	1: 500,000	1: 700,000	1: 900,000	
	min.	min.	min.	min.
A. Tubes inverted after 6 hours incubation...	310	304	298	308
B. Tubes inverted hourly.....	238	182	138	187

[1]Reduction times represent the average of two separate lots of dye. For the majority of samples, duplicate tubes were run at each dye concentration.

should be pointed out that the samples in Section A were an entirely different lot from those in Section B, so that the results in these two sections cannot be directly compared.

In Section A there is evidence of a slight retardation of reduction time as the dye concentration is increased but scarcely enough to be regarded as significant. In Section B, however, a much more marked influence is apparent. In both sections there is excellent agreement between the results with the two different salts at the same concentration (1:700,000).

At the time it was felt that these data suggested that it was not desirable to redistribute the bacteria by inverting the contents of the tubes at hourly intervals. However, recent studies on a much more extensive scale have failed to confirm these findings.

These studies indicated that there was every reason to believe that methylene blue thiocyanate might advantageously be substituted for the chloride at present employed, but did not settle the question as to the optimum concentration of dye for use in the test.

A COMPARISON OF MILK AGAR AND STANDARD AGAR FOR MILK ANALYSIS

With a growing realization of the shortcomings of the standard method for plate count determinations on milk, many workers have been seeking ways and means of improving this technique. Studies by this Division in 1932, indicating that a lower incubation temperature and the addition of skim-milk to the agar greatly increased the count, have since been extended and confirmed in England and the United States. Since any change in the present technique would affect existing bacteria count standards, extensive tests on any modified technique are desirable. At the request of the Committee on Laboratory Methods of the International Association of Dairy and Milk Inspectors, plate counts for pasteurized milk on standard agar were compared with counts on the same agar containing 0·5 per cent skim-milk. All plates were incubated at 32° C., the temperature which workers at the Geneva, N.Y., station have found to yield the maximum count with minimum variability due to slight changes in temperature during incubation.

The results on a series of 45 samples indicated very clearly that the addition of skim-milk greatly improved the nutritive value of the agar. Eleven of the 45 samples gave counts on the milk agar more than double the counts on standard agar, the most striking increase being from 7,000 on standard agar to 106,000 on milk agar. In addition, the milk agar yielded appreciably larger colonies, while counts on different dilutions never showed the serious discrepancies occasionally encountered with standard agar.

METHODS FOR REDUCING CONTAMINATION FROM THE CREAM SEPARATOR

Contamination from improperly cared for separators is undoubtedly a major factor in lowering the grade of cream for buttermaking. Where only a small amount of milk is separated, the work involved in washing the separator after each milking often appears to the farmer to be greater than is economically justifiable. While recognizing that best results can be obtained only where the separator is thoroughly washed and sterilized after each separation, it seemed worth while to study the effectiveness of simpler methods of caring for farm separators in the hope that the present degree of contamination might be reduced appreciably.[1]

In an unwashed separator the bacteria present in the milk residue and slime find ample food and moisture for growth. At ordinary temperatures multiplication proceeds rapidly and the cream picks up large numbers of bacteria at the following separation. This growth could be prevented by holding the bowl at a low temperature but this is rarely available to the smaller cream producer. Growth might also be prevented by leaving the bowl filled with some bacteriostatic solution, provided one could be found which was noncorrosive and harmless. Aside from this, the only practicable steps appear to be to flush the bowl thoroughly with water to remove as much bacterial food as possible, and to flush again before the next milking, preferably with a hypochlorite solution.

In the first series of tests (November-December, 1934) the bowl was flushed after separation with 0·5 per cent solution of Metso (sodium metasilicate) which was left in the bowl overnight. While entirely effective in restraining bacterial growth, corrosion of the bowl and disks was so marked within a few days that the treatment had to be discontinued.

In the next series of tests the bowl was flushed with two gallons of cold water (40° C.) after the evening's and before the morning's separation, while the bowl was drained and held in an inverted position for 14 hours at approximately 25° C. (77° F.). Counts on the cream from a separator so treated averaged 101,800 per ml., while the original milk showed less than 5,000 per ml. When a quick acting hypochlorite at 50 p.p.m. was substituted for cold water in flushing out the bowl before the morning separation the count averaged 5,890 per ml. from milk averaging 5,210 per ml.

Further detailed studies were made during April and May, 1935, in which the hypochlorite rinse was compared against cold water, and both methods compared against washing and steaming after each milking. Portions of the data obtained are summarized in table 4. Where the bowl was held overnight at temperatures between 12·2 and 22° C. (54 to 71·6° F.) no significant contamination was encountered with any of the three methods, but when the holding temperature was raised to 28° C. (82·4° F.) to simulate hot weather conditions, there was a marked increase in bacterial contamination.

The exact evaluation of the relative efficiency of the various separator treatments is complicated by the striking variations in the keeping time of the milk and cream, and by the fact that morning's milk invariably showed counts two to three times as high as those for evening's milk. However, one may safely conclude that the temperature at which the unwashed bowl is held overnight is the most important single factor governing the degree of contamination of the cream at the next separation. Where this does not exceed 20° C. (68° F.) and where the bowl is thoroughly flushed out after the evening's and before the morning's separation, contamination is slight. Where the bowl is held at 25 to 28° C. (77-82·4° F.) bacterial growth is so enormous that large numbers contaminate the cream in spite of thorough flushing. Flushing with hypochlorite solution instead of cold water before the morning separation results in a significant reduction in bacterial contamination at either holding temperature.

[1] For further details see Johns, C. K. Sci. Agr. 16: 373-390. 1936.

TABLE 4.—EFFECT OF VARIOUS SEPARATOR TREATMENTS ON BACTERIAL COUNT AND KEEPING TIME OF CREAM HELD AT 15·6°C. (60°F.)

Separator treatment	Bowl held	Plate count per ml.	Keeping time, hours	Percentage of tests showing coliform bacteria		
				1 ml.	0·1 ml.	0·01 ml.
Washed and steamed	12·2—25·6°C. (54—78°F.) 14 hours	4,600	80·5	75·0	0·0	0·0
Flushed with cold water after P.M. and before A.M. run.	28°C. (82·4°F.) 14 hours	100,000	61	100·0	66·7	0·0
	12·2—22°C. (54—71·6°F.) 14 hours	9,390	75·9	28·6	0·0	0·0
Flushed with cold water after P.M. run, with hypochlorite before A.M. run.	28°C. (82·4°F.) 14 hours	23,800	73·7	66·7	50·0	33·3
	12·2—22°C. (54—71·6°F.) 14 hours	2,680	75·8	0·0	0·0	0·0
	12·2—25·6°C. (54—78°F.) 14 hours	1,890	78·8	75·0	25·0	0·0

Comparisons of the keeping time of evening's and morning's cream showed a difference in favour of the evening's cream. Where eleven samples of similar bacterial content were compared, the average values were 85·1 hours for evening's and 77·7 hours for morning's cream. A similar but slighter difference was noted where the corresponding milk samples were compared.

Since thick cream keeps better than thin, it was rather surprising to find that in every case but one the original milk showed on an average a definitely longer keeping time than the cream separated from it, despite the lower count of the cream. A comparison of 20 pairs of samples (average count 1,965) gave values for keeping time of 87·4 and 81·7 hours respectively. Further studies revealed that the growth rate in cream is generally higher than in milk while an increase in titratable acidity appears approximately ten hours earlier with cream.

METHODS FOR THE DIAGNOSIS OF CHRONIC CONTAGIOUS MASTITIS

The growing recognition of the importance of mastitis to the dairy industry, and of the need for definite measures to control this widespread infection, has focused attention upon methods of diagnosis. Farmers, veterinarians and others have too often regarded the diagnosis of this disease as something comparable to the diagnosis of tuberculosis or contagious abortion, where a single biological test usually tells whether or not the animal is infected. Unfortunately no single test has so far been developed (nor is there much likelihood that one will be) which will detect mastitis with the same degree of accuracy as the tuberculin test detects bovine tuberculosis, for the problem is far more complex.

The word mastitis means "inflammation of the udder." Inflammation of the udder may be caused by any one of a number of bacterial species or by non-bacterial agents such as bruises, etc. If the mastitis problem concerned only cases where the udder was obviously inflamed, there would be little need for special diagnostic methods. The term "mastitis" however has a much wider meaning in actual practice, being generally applied to any udder definitely infected with organisms known to produce inflammatory conditions. It is generally believed that the bulk of mastitis is due to infection with *Streptococcus*

agalactiae. This organism attacks the secreting tissue, changing it to fibrous or scar tissue. In most animals there is an occasional "flare-up" in which the udder appears inflamed, while the secretion is often purulent or flaky. When this subsides and the milk again appears to be normal the animal is commonly regarded as being cured. However, the infection persists and the progressive change of the secreting tissue to fibrotic tissue goes on until the hardened areas can readily be recognized by anyone. The result of this destruction of secreting tissue is of course reflected in decreased milk production, while in most cases large numbers of streptococci are shed in the milk, which may be so changed in composition as to give rise to difficulties in cheesemaking and other processes.

Since it is generally believed that this is the only contagious type of mastitis, adequate control and eventual elimination of this type of mastitis require the detection of all infected cows and their segregation or removal from the non-infected animals. This, however, is not readily accomplished because of the intermittent manner in which the organism is frequently shed and means that repeated sampling is necessary, involving much time and effort. Furthermore, the organism is frequently accompanied by other types of bacteria and may be overgrown by them during incubation. Definite diagnosis requires the isolation of the streptococcus and study of a number of biochemical and cultural characteristics in order to identify it, so that obviously this method is much too expensive for general application.

Milk from infected quarters is generally changed in composition to a greater or lesser degree. Based upon this fact, a large number of simple tests have been developed and their use advocated as means of diagnosis. Unfortunately the limitations of these indirect tests are not always appreciated by those using them, and it is quite likely that many animals have been wrongly condemned as "infected" as a result of their indiscriminate use. Changes in the composition of the milk may result from non-bacterial causes, or may be caused by other bacteria which do not appear to be contagious, while in many cases the reasons for the changed character of the secretion remain unknown.

In October 1935, a program was initiated for the control of chronic contagious mastitis in the Central Experimental Farm dairy herd, based upon the detection of infection with *Str. agalactiae*. A large number of methods recommended by various workers were tried out, together with modifications developed in these laboratories. The method finally adopted for routine control was to take a composite sample from the four quarters of each cow at the evening milking. A catalase test,[1] using the Hastings tube method, was set up at once and read next morning. The remainder of the sample was held overnight at 5°C. Next morning Burri slants of sucrose-tryptone agar or Bowers and Hucker agar were inoculated with a 6 mm. loopful from the cream layer, and colonies resembling streptococci fished to æsculin broth. Those failing to split æsculin were then seeded into litmus milk, methylene blue milk (1:5,000) and triple sugar broth mannital, inulin, raffinose) for identification. Where catalase values of over 30 per cent were encountered, individual quarter samples were taken at the first opportunity and subjected to a similar analysis. In this way more time is available for the study of animals giving abnormal milk than where individual quarter samples are taken throughout.

Quite early in these studies it was noted that approximately one-third of the animals in the herd gave abnormal biochemical reactions (catalase, chlorides, pH) without any streptococci being demonstrated on repeated samplings. A number of these were first-calf heifers, and several of them were selected for more detailed study. The procedure adopted was to take occasional series of samples at from 5 to 40 consecutive milkings, as only in this way was it possible to obtain a comprehensive picture of the condition of the quarter in question.

[1]The catalase test has been found to be the most satisfactory of the various indirect tests studied. The differences between milk from normal and abnormal quarters are greater than with other tests, while it indicates infection at an earlier stage than do the other tests employed.

(See Figure 2). In a number of instances marked rhythmic fluctuations in the biochemical reactions of the foremilk were noted; in some cases high values were regularly noted for the evening milking with lower values for the morning milking. Fluctuations in bacterial numbers likewise frequently showed a regular rhythm, varying inversely with the biochemical values, a high count at the morning milking coinciding with low biochemical values followed by a low count and high biochemical values. No one species appeared to be associated with these abnormalities in secretion, the predominant types being indistiguishable from those appearing on plates from normal udders. No clinical evidence of mastitis was ever noted while the whole of the milk obtained at a given milking would be judged as normal in composition. The reason for these abnormalities in secretion has yet to be found, although some evidence was obtained that their appearance was preceded by a marked rise in the general level of bacterial counts.

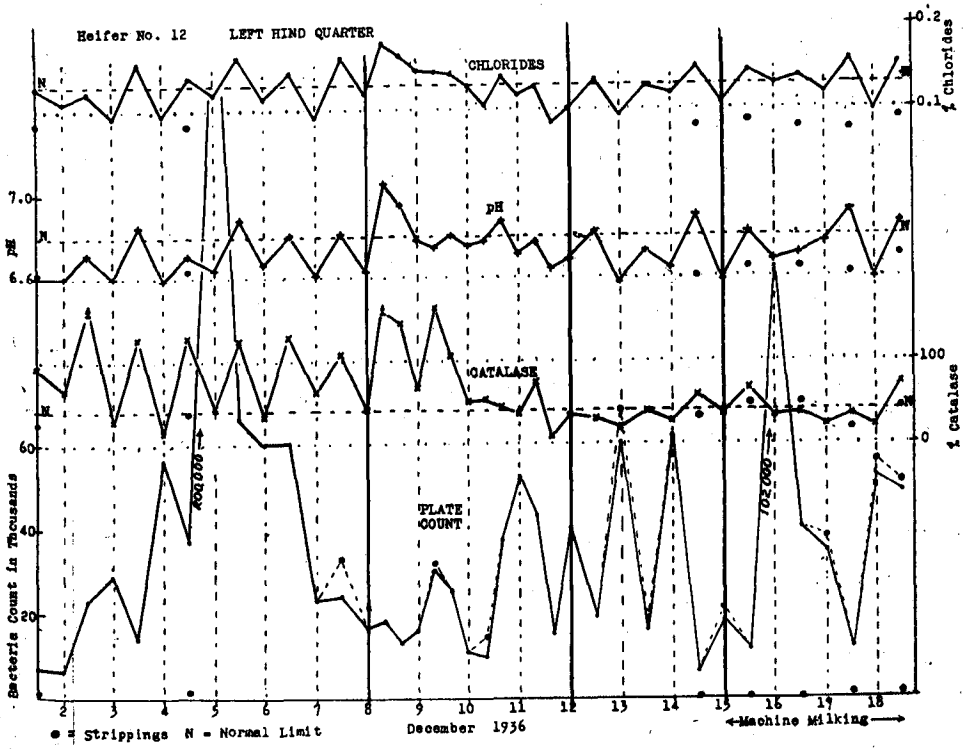

FIG. 2.—Illustrating fluctuations in biochemical values and plate counts of foremilk from an apparently normal, streptococcus-free quarter.

While these findings should not be construed as condemning the use of indirect biochemical tests as aids in the detection of mastitis, it cannot be too strongly emphasized that they cannot be depended upon by themselves to afford a definite diagnosis. Examination for *Str. agalatiae* is essential, and in many cases a satisfactory diagnosis can be made only when all possible information, clinical, bacteriological and biochemical, is obtained and studied in the light of the history of the animal.

DAIRY CONTROL WORK

In addition to the investigatory 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 in 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.

To assist the Division of Animal Husbandry in the control of mastitis in the dairy herd, periodic examinations were made of all cows for infection by *Str. agalactiae*. According to the findings the animals were segregated into definitely infected, suspicious and non-infected groups, which permitted of better control in preventing the spread of the disease and in aiding its elimination.

SOIL MICROBIOLOGY

LEGUME CULTURE DISTRIBUTION

The distribution of cultures for the inoculation of legume seed to Canadian farmers was continued during the years 1934-36. The distribution of "nitrocultures" was maintained for educational and experimental purposes, sufficient culture for the treatment of 60 pounds of legume seed being sent free to any farmer on the understanding that the user report the results.[1] The object was therefore (*a*) to encourage the more widespread practice of seed inoculation and (*b*) to accumulate the data on the practical results of seed treatment under field conditions. During the years under review the numbers of cultures distributed were 4,184, 5,407 and 5,867 respectively, making a total of 15,458 for the period. Of these, approximately 2,800 were sent to the Prairie Provinces in co-operation with the program of Government aid to farmers in drought areas.

RESULTS OF INOCULATION EXPERIMENTS CONDUCTED BY FARMERS

By means of reports returned by farmers receiving cultures for legume inoculation, the Division has been accumulating information over a period of 12 years on the practical benefits of seed treatment as judged by the farmers themselves, whose opinion will largely determine the value of any agricultural practice. A summary of reports received to the end of 1936 is presented in table 5, comprising data from 2,475 reports.

TABLE 5.—SUMMARY OF REPORTS OF LEGUME INOCULATION BY FARMERS

—	Alfalfa	Sweet clover	Red clover	Alsike clover	Peas	Vetches	Beans	Soy-beans	Lupines	Total
Total reports received	1,449	399	271	74	90	10	53	120	9	2,475
Benefit from inoculation	1,195	294	215	65	67	8	44	82	8	1,978
Percentage benefit	82·4	73·7	79·3	87·8	74·4	80·0	83·0	68·3	88·9	79·9
Legume grown for first time	1,183	311	130	40	53	6	37	106	8	1,886
Benefit from inoculation	1,007	234	107	36	36	5	26	78	7	1,524
Percentage benefit	85·1	75·2	82·3	90·0	67·9	83·3	70·3	73·6	83·8	80·8
Cases reporting no benefit	254	105	56	9	23	2	9	38	1	497
Treated and untreated crops grew well	137	36	20	2	9	2	3	21	–	230
"No catch" reported	38	17	13	1	3	–	3	1	–	76
Winter killed	3	4	1	–	–	–	–	–	–	8
Drought	26	24	12	3	6	–	1	1	–	73
Blown out	1	1	–	–	–	–	–	–	–	2
No reason given	49	23	10	3	5	–	2	15	1	108

From the table it is observed that the results are distinctly favourable to inoculation. There is reason to believe that not only in the case of alfalfa, but also for common clovers and other legumes, seed treatment may be a very useful practice. In approximately one-half of the cases where no benefit was noted, both treated and untreated crops grew well, indicating that the soil was already supplied with suitable bacteria rather than a failure of the culture. Although the percentage of favourable reports was slightly higher where legumes were grown for the first time, information from reports indicated that re-inoculation may be of value. Greenhouse tests, supplemented by field trials at Beaver-

[1] The general distribution was discontinued in 1937, satisfactory commercial cultures now being available.

lodge, have shown that different "strains" of legume bacteria may vary greatly in nitrogen-fixing capacity and so in their ability to aid a crop. The addition of a "good strain" of bacteria rather than the mere presence of nodule-forming bacteria in the soil is believed to be an important factor which explains the value of re-inoculation. (See figure 3.)

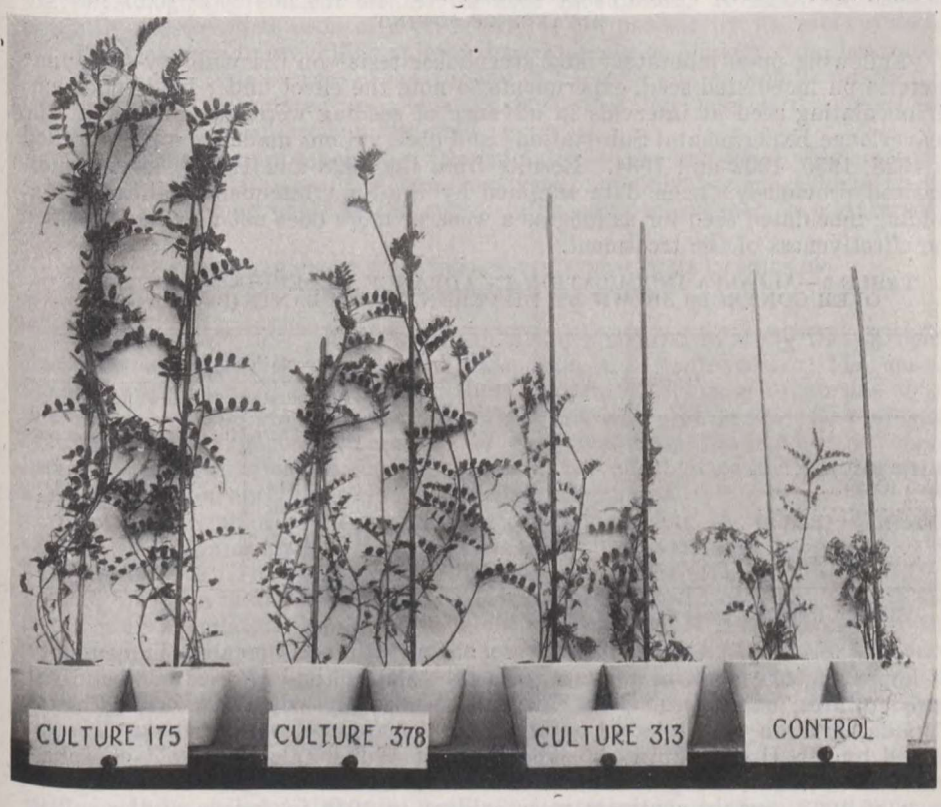

FIG. 3.—Variation in efficiency shown by different strains of nitrogen-fixing bacteria *(Rhizobium leguminosarum)* for vetch.

EXAMINATION OF MISCELLANEOUS INOCULANTS

During the three-year period a number of legume inoculants appearing on the market were examined. The tests undertaken consisted in estimating the number of organisms in the culture and in making greenhouse tests to note the ability of the culture to produce nodules on the appropriate legume plants when applied as directed. Seeds were sown in sterile sand to which was added plant food minus nitrogen with controls of uninoculated seed and of seed inoculated with a pure culture of *Rhizobium* corresponding to that of the inoculant.

Legume inoculants available to farmers in Canada are now believed to be generally reliable. Though various types are available, there is nothing to indicate that the dry method, in which the culture, in the form of a powder, is mixed dry with the seed, is to be preferred to the older method whereby the seed is moistened when the culture is applied. Moreover, inoculants containing bacteria for but one cross-inoculation group are believed better than those containing bacteria for two or more legume groups, since tests have shown that in mixed cultures one strain may become ineffective.

Cultures purporting to aid the growth of all types of crops, non-legumes as well as legumes, reappeared in 1936. As no evidence was forthcoming of the practical worth of such preparations they were kept from sale. Such "all-crop" inoculants are not to be recommended.

ALFALFA INOCULATION UNDER FIELD CONDITIONS. EFFECT OF INOCULATING SEED IN ADVANCE OF SOWING

Following upon laboratory and greenhouse tests[1] on the viability of legume bacteria on inoculated seed, experiments to note the effect under field conditions of inoculating seed at intervals in advance of seeding were inaugurated at the Beaverlodge Experimental Sub-station[2] and observations made from plots seeded in 1928, 1930, 1932 and 1934. Results from the 1928 and 1930 seedings, summarized previously[3] from data supplied by the Superintendent, indicated that holding inoculated seed for as long as a week or more does not necessarily lessen the effectiveness of the treatment.

TABLE 6.—ALFALFA INOCULATION IN ADVANCE OF SEEDING. INCREASE OVER CONTROLS SHOWN BY DIFFERENT INOCULANTS (BEAVERLODGE)[1]

Inoculant	Inoculation in advance of seeding		
	Immediate	24 hours	1 week
	lb. per acre	lb. per acre	lb. per acre
No. 173 (Ott.)	560	369	1,235
Mixed (Ott.)	1,664	1,840	1,636
B.L. 3	1,207	1,240	1,112
Acid tolerant (Roth.)	1,342	1,971	2,162
Soil	1,082	1,992	954
Aver. (all methods)	1,171	1,482	1,420

[1]1935 crop, from 1934 seeding.

1932 Seeding.—Alfalfa plots were sown with seed treated immediately, 24 hours and one week in advance, using the soil method of treatment and two pure cultures, one (strain No. 173) used previously with success in Eastern Canada, and an acid-tolerant strain obtained from Rothamsted, kindly furnished by Dr. H. G. Thornton. The effect of adding calcium diacid phosphate was also tested. Though no differences were evident the year of seeding and most of 1933, certain contrasts were evident in 1934 in favour of the culture inoculated plots, with the acid-tolerant culture showing to best advantage. As the stands were patchy, due to unfavourable conditions at seeding and to ice-killing, yields were not taken. The interval of inoculation in advance of seeding seemed to have no pronounced effect nor did the use of calcium diacid phosphate.

1934 Seeding.—A similar seeding was made in 1934, in which, in addition to soil inoculation, four cultures were used (No. 173; Ottawa mixed strains; culture BL3, isolated from Beaverlodge soil; Rothamsted acid-tolerant strain). Duplicate sets were sown, inoculated immediately, 24 hours and one week in advance. Though little difference between the inoculants was evident in 1934, contrasts appeared in 1935. Though all methods of inoculation were superior to the uninoculated controls, Rothamsted acid-tolerant, Ottawa mixed and BL3 were definitely better than No. 173 and the soil inoculated plots. Owing to the nature of the land the location was most favourable to the one week interval plots and least favourable to the "immediate" series. The yields, obtained at harvest in 1935, are recalculated as increase over controls in each pre-treatment series, and summarized in table 6.

[1]Reports of Dominion Agricultural Bacteriologist for 1925 and for 1926.
[2]For detailed results see Reports of Superintendent, Dominion Exp. Sub-station, Beaverlodge, Alta. from 1929 to 1936.
[3]Progress Report of Dominion Agricultural Bacteriologist for 1931-33. Dom. of Canada, Dept. of Agr. 1934.

Reviewing the whole series of field tests from 1928 to 1934 the results are of much practical significance in legume cultivation. From the mass of data three main conclusions may be drawn:—

(1) Different cultures of legume nodule bacteria may vary in their nitrogen-fixing capacity under field conditions. This indicates the importance of employing physiologically efficient strains for seed inoculation. A strain of bacteria normally present in the soil of a given area is not necessarily the most efficient.

(2) Contrary to prevailing opinion, based largely on analogy from laboratory and greenhouse tests on rate of destruction of bacteria and on nodule production, the holding of inoculated seed several days does not necessarily lessen the effectiveness of inoculation in the field.

(3) Inoculation may show a delayed action. The beneficial effect of treatment may not be apparent the first crop year, but only become evident in the second or succeeding years as the organism becomes established in certain soils.

A FOUR-YEAR STUDY OF NITROGEN-FIXING BACTERIA IN SOILS OF DIFFERENT FERTILIZER TREATMENT

In November, 1931, an investigation was commenced to study the nitrogen-fixing bacteria in soils, particularly *Rhizobium* and *Azotobacter*. The special purpose was to note the incidence of these important groups of organisms as influenced by season, cropping and fertilizer treatment. While the effect of such factors upon the general microflora of soils has been the subject of much investigation, less attention has been paid to the effect of season and management practices on specific groups of soil micro-organisms.

Both the symbiotic and non-symbiotic nitrogen-fixing bacteria are regarded as groups of organisms of definite significance in the maintenance of fertility in most arable soils. The persistence of different cross-inoculation groups of nodule-forming bacteria is of importance in relation to the growth of legume crops and need for re-inoculation, while non-symbiotic fixation must be influenced by the numbers of organisms as they react to their environment. The study here described [1] is an attempt to follow for four consecutive years the numbers of three species of *Rhizobium* and of *Azotobacter* under definite conditions of cropping and fertilizer treatment

Numbers of *Rhizobium trifolii*, *Rh. leguminosarum*, *Rh. meliloti* and *Azotobacter* were determined at four-week intervals from November, 1931, to October, 1935, in three soils of different fertilizer treatment supporting a rotation of mangels, oats, clover and timothy. For 20 years the soils had been receiving respectively no fertilizer, farmyard manure and artificial fertilizers. They contained, at the start, 0·113 per cent, 0·162 per cent and 0·126 per cent nitrogen respectively and judged by crop yields represented soils with striking differences in fertility.

Numbers of *Rhizobium* were determined by a method in which increasing dilutions of soil were added to sterile soil-sand in small crocks of 250 ml. capacity, in which sterilized seeds of alfalfa, red clover and vetch were placed. Examination of the seedlings after two or three weeks in the greenhouse permitted an estimate of the numbers of the three species of *Rhizobium* according to the nodulation of the plants in the dilution series. Numbers of *Azotobacter* were estimated by an adaptation of the agar plate method, in which 0·3 gm. of soil was scattered on the surface of modified Ashby's agar, 10 plates per sample. In addition total numbers of bacteria were estimated by regular plating methods.

Results and Discussion.—Data from 55 consecutive analyses are summarized in table 7. The results indicate that the three species of *Rhizobium* studied not only varied in their actual abundance, but also showed relative differences varying

[1] For a more detailed presentation see Lochhead, A. G. and Thexton, R. H. Can. J. Research, C. 14: 166-177. 1936.

with the soil. Thus in the case of *Rh. trifolii* there is comparatively little difference in the capacity of the three soils to support this organism. In the first year following the crop of clover the manured area gave higher average counts, but in the following years less difference was noted between the soils In the case of *Rh. leguminosarum* it is plain that soil N (no fertilizer) supports a much lower population than either of the soils receiving fertilizer. Thus soil X (manure) contained approximately 21 times as many, and soil Y (artificial fertilizer) 25 times as many organisms as soil N, considering averages for the four-year period. Similarly, with *Rh. meliloti* the two fertilizer areas, X and Y, were found to support respectively ten and six times as many organisms for the four-year period as soil N.

Rhizobium trifolii was present in all three soils in much greater numbers than the other legume bacteria studied. As it was the only species with its host

TABLE 7.—SUMMARY OF COUNTS OF BACTERIA IN SOILS AT FOUR-WEEK INTERVALS FROM NOVEMBER, 1931 TO OCTOBER, 1935

Soil and year	Bacteria per gram of soil (log. average)				
	Rh. trifolii	*Rh. leguminosarum*	*Rh. meliloti*	*Azotobacter*	Total count
SOIL N (NO FERTILIZER)—					
First year after clover	379,000	235	130	100	–
Second year after clover	161,000	155	155	113	13,700,000
Third year after clover	67,000	170	165	172	30,100,000
Clover crop	226,000	155	295	130	23,400,000
Average for 4 years	174,000	175	176	123	21,500,000
Average when soil frozen	186,000	140	120	134	25,000,000
SOIL X (MANURE)—					
First year after clover	1,119,000	6,320	990	37	–
Second year after clover	158,000	2,690	1,160	61	22,400,000
Third year after clover	131,000	2,400	2,330	64	31,100,000
Clover crop	345,000	3,710	3,160	79	32,900,000
Average for 4 years	310,000	3,600	1,690	55	28,300,000
Average when soil frozen	230,000	3,650	1,360	58	33,500,000
SOIL Y (ARTIFICIAL FERTILIZER)—					
First year after clover	370,000	7,360	590	59	–
Second year after clover	90,000	2,540	910	56	27,600,000
Third year after clover	104,000	3,360	720	67	33,400,000
Clover crop	293,000	5,370	2,810	76	31,200,000
Average for 4 years	184,000	4,340	1,000	64	30,800,000
Average when soil frozen	156,000	5,890	890	69	39,200,000

plant in the crop rotation, its greater abundance during the year following clover might well be expected. After the first year further decline was slight or absent, however, the numbers remaining at a comparatively high level and suggesting that the three soils are capable of supporting a much higher population of *Rh. trifolii* than of *Rh. leguminosarum* or *Rh. meliloti*. Table 8 shows the average relative numbers for the second and third years following clover as affected by soil fertility. Soil N, with no fertilizer for over 20 years, was relatively much less suited to *Rh. leguminosarum* and *Rh. meliloti* than to *Rh. trifolii*.

TABLE 8.—RELATIVE NUMBERS OF SPECIES OF *RHIZOBIUM*

—	*Rh. trifolii*	*Rh. leguminosarum*	*Rh. meliloti*
Soil N (no fertilizer)	1,000	1·6	1·6
Soil X (manure)	1,000	18	12
Soil Y (artificial)	1,000	30	8

The effect of its symbiont on the numbers of *Rh. trifolii* was noted during the year following clover (1932), the crop being ploughed under that spring preparatory to sowing timothy. The numbers remained more or less constant the second and third years (1933 and 1934) though in the spring of 1934 clover was sown with oats. During that year the plants did not develop to more than two or three inches in height, and no noticeable effect was found on the average numbers of nodule organisms in the soil. Only with the spring and summer of 1935, when the clover crop developed, was the presence of the symbiont reflected by a general rise in numbers of *Rh. trifolii*.

Apart from the effect of the recurrent growth of clover on *Rh. trifolii*, the study did not indicate any significant effect of the crops upon the numbers of the nodule organisms studied, nor did the moisture content of the soil or the season of the year appear to be of significance. Previous studies[1] on the effect of frost on the general microflora of soil had shown that after more than three months' freezing, bacterial numbers remained at normal levels. Data from the present work indicate that the legume nodule bacteria in the soils studied are to be regarded as cold tolerant, i.e., able to withstand the prolonged period of continuous freezing with relatively little or no diminution in numbers. This ability to tolerate continued frost is believed to be an important factor in the maintenance of successful legume cultivation in regions with severe winters.

The results of the determinations of *Azotobacter* are also shown in table 7. It is noted that soil N, without fertilizer for more than 20 years, and giving much lower crop yields than soils X and Y, showed consistently the highest numbers. The depressing effect of farmyard manure and nitrate fertilizer respectively is in agreement with observations of a number of workers who noted a reduction in the growth of *Azotobacter* from the application of nitrogenous fertilizers.

A definite seasonal fluctuation in numbers of *Azotobacter* may be observed from fig. 4, showing the four-year monthly averages for the plate counts. In all soils, minimum numbers were found in July, with maximum numbers in March, in which month samples were all taken in the latter half, after the first thawing had occurred. This rise in numbers immediately following the period of frost was previously noted in a study of the general bacterial soil flora. The data do not indicate any depressing effect of cold, however, the average counts being well maintained during the winter months.

A striking feature of the present study is the generally low number of *Azotobacter* in soils as indicated by any cultural method of enumeration. When one finds a few hundreds or at most a few thousands in a gram of soil which may harbour a bacterial population of one hundred million in addition to numerous fungi, protozoa, etc., one may reasonably doubt the importance of *Azotobacter* as an agent of nitrogen fixation under field conditions. It is possible that our counting methods do not give a reasonable estimate of *Azotobacter* numbers in soil, and further development of such methods is desirable in order to judge the role played by the organism in field soils. The presence in soil of numerous other aerobic organisms to which capacity for nitrogen fixation has

[1] Report of the Dominion Agricultural Bacteriologist for the year 1924, p. 12-16. 1925.

been ascribed, and more particularly, the possible importance of anaerobic bacteria as agents of nitrogen fixation under practical conditions lead to the belief that the part played by *Azotobacter* is an open question.

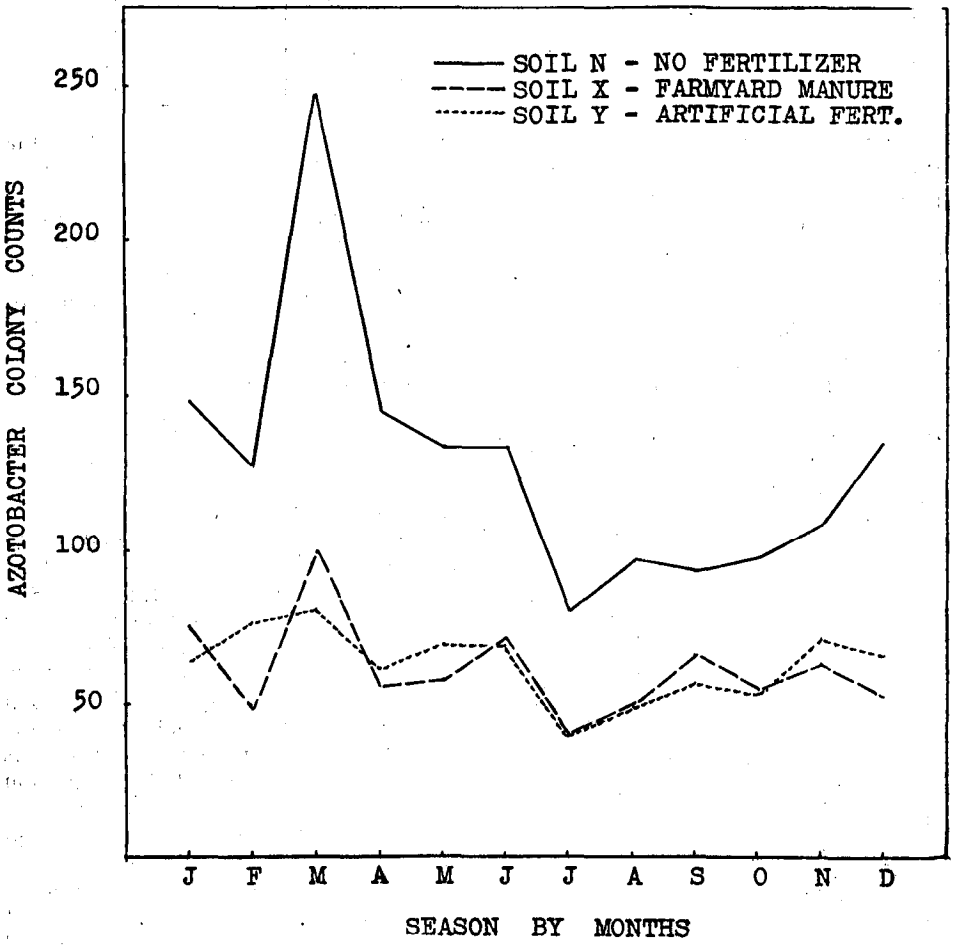

FIG. 4.—Seasonal variation of *Azotobacter* in soils of different fertilizer treatment. Averages for four years.

Data from the total counts seen in table 7, do not indicate marked differences between the soils studied. Somewhat higher average numbers were obtained from the two fertilized areas, X and Y, than from the unfertilized area, with numbers well maintained when the soil was frozen. As noted in other investigations with Canadian soils, a certain seasonal trend was apparent, with generally lower numbers from July to September following higher numbers in spring. The data indicate, however, that as an index of specific physiological groups of organisms in soil, such as nitrogen-fixing types, the total count method is unreliable. Its application to problems of soil fertility, then, must be considered as very limited.

Micro-organisms and Crop Yields.—A comparison between the relative productivity of the three areas studied, and the average values for the bacterial

counts are given in table 9. Relative average yields for the different crops during the period of the test are given as well as relative counts (logarithms) for the various groups of micro-organisms.

TABLE 9.—RELATIVE YIELDS AND NUMBERS OF BACTERIA

Crop	Soil area	Relative values					
		Crop yield	*Rh. trifolii*	*Rh. leguminosarum*	*Rh. meliloti*	*Azotobacter*	Total count
Timothy	N	100	100	100	100	100	-
	X	164	109	161	153	77	-
	Y	134	99	167	142	87	-
Mangels	N	100	100	100	100	100	100
	X	1,070	100	157	140	87	103
	Y	950	95	156	135	85	104
Oats	N	100	100	100	100	100	100
	X	138	106	152	152	81	100
	Y	129	104	159	149	83	101
Clover	N	100	100	100	100	100	100
	X	239	104	163	142	90	102
	Y	185	102	170	139	88	101

The relative productivity varies with the crop, the difference between the unfertilized and fertilized areas being most emphasized with mangels and less with the other crops. Although this pronounced difference with the hoed crop is not reflected in the bacterial counts, yet the relation of productivity to micro-population is of interest.

With total count, *Azotobacter* count, and *Rh. trifolii* count, no relation to productivity can be noted. The numbers of *Rh. leguminosarum* and *Rh. meliloti* show distinctly higher values when the fertilized areas are compared with the less productive area, N. Only in the case of *Rh. meliloti*, however, do the numbers found coincide in order with the order of productivity of all three soils for all four crops. It is suggested from the findings that study of the abundance of certain species shows greater promise of providing a reliable index of soil fertility than the numbers of micro-organisms as a whole.

BACTERIOLOGICAL STUDY OF MULCHES FOR ORCHARD SOILS

In co-operation with the Divisions of Horticulture and Chemistry, studies were carried on in 1935 and 1936 to note the effect of different mulches on the nitrogen content of orchard soils. Samples of soil were taken regularly throughout the season at different depths from four areas, with different surface treatment as follows: (a) hen manure mulch, (b) sod with hay mulch, (c) sod alone and (d) clean cultivation.

As judged by the total numbers of bacteria, actinomyces and fungi, soil under hen manure mulch was found to be the most active biologically, particularly in the first few inches. Soil under sod with hay mulch came second, followed in order by soil under sod and cultivated soil. This order coincided with that for organic nitrogen content. Cultivated soil, however, showed much less variation with depth than the three other soils, the numbers of organisms in the latter decreasing notably with depth, particularly fungi.

In both seasons nitrifying bacteria were most numerous under hen manure mulch and least abundant under sod alone, at both 0–2 inches and 2–6 inches. Considerable variation in numbers of nitrifying organisms was found throughout the season, with highest counts generally in July and October and greater fluctua-

tions at the higher levels. The "nitrifying capacity" of the soil, as measured by production of nitrate from ammonium sulphate, showed a tendency to increase towards the latter part of the season, and showed a fair measure of correlation with the amount of nitrate- nitrogen in the soils. Soil under sod alone displayed the lowest nitrifying capacity during both seasons, with less difference between the other soils.

Wide variations in moisture content throughout the season tended to confuse relationships. The nitrate content of the soils varied in general, inversely with the moisture, whereas a more direct correlation was observed between the latter and the abundance of nitrifying organisms. In addition to the leaching influence of excessive soil moisture, variation in nitrate absorption by orchard trees during the season complicates correlation of soil nitrate accumulation with the measurement of bacterial numbers or their activity.

Tests on the incidence of nitrogen-fixing bacteria (*Azotobacter*) showed the absence of this group of organisms from the soil under hen manure mulch during both seasons, though they were present in the other three soils. The depressing effect of manures on *Azotobacter* is in agreement with findings reported in a previous investigation (see p. 19). In the three soils containing *Azotobacter* the organism was found at all depths to 24 inches, occurring more abundantly in the soils covered with sod than in the cultivated soil. In the former soils maximum numbers were found at 6 – 12 inches, while in the cultivated soil differences were less pronounced.

QUALITATIVE STUDIES OF SOIL MICRO-ORGANISMS

In September, 1936, a program of research was inaugurated dealing with fundamental aspects of soil microbiology, with special attention to the qualitative nature of soil micro-organisms. The limitations of any purely quantitative estimation of bacteria, fungi, etc., in soil and the inadequacy of our knowledge of their qualitative nature indicate the need for investigations on the types of micro-organisms and their as yet little known functions. It is felt that such studies should aid, not only in adding to our knowledge of soil and the part played by micro-organisms in soil fertility, but also help problems such as the relationship of normal soil micro-organisms to soil-borne diseases. The progress of the investigations, commenced towards the close of the period here reported, will be indicated in later reports.

FOOD BACTERIOLOGY

FROZEN VEGETABLES AND FRUITS

In 1934 a series of experiments was commenced in co-operation with the Division of Horticulture to study the microbiological factors concerned with the preparation and handling of frozen pack vegetables and fruits. A knowledge of the effect of freezing and defrosting on micro-organisms is important, not only from the public health standpoint, but also from the standpoint of deterioration and loss of keeping quality of the products themselves.

Studies, described in detail elsewhere[1], were made of products packed during the seasons of 1934, 1935, and 1936, including asparagus, spinach, peas, beans, corn, strawberries and raspberries. The products were stored in a freezer at −17.8°C. (0°F.) and investigations made of the influence of different methods of preparation and packing, the effect of freezing on numbers and types of organisms surviving during storage and the growth of organisms after defrosting.

Effect of Freezing on Numbers of Micro-organisms.—Determinations of bacteria, moulds and yeasts in frozen products at regular intervals up to nine

[1] Loehnead, A. G. and Jones, A. H. Food Research, 1: 29-39. 1936.

months, made with the 1934 and 1935 packs, gave similar findings. The data obtained indicated that great variations may be encountered in the microbial content of freshly packed products. On account of variations in the supply of raw material and daily fluctuations in dust and soil conditions, the bacterial load of the raw product may be extremely variable. The findings showed, however, that differences in original microbic content of freshly packed material tend to become largely obliterated during storage.

As far as methods of packing are concerned, the experiments did not indicate that they have any significant effect on the germs which survive during storage of the vegetables. Comparison of water and brine packs showed that differences in bacterial content present in freshly packed material became less pronounced during storage, so that little distinction is possible between the two methods. The only exception noted was corn where considerably larger proportions of bacteria were found to survive with the water pack than with the brine pack. Likewise, little significant difference was noted between the numbers of surviving organisms in the dry and wet packs. Only in the case of peas were there considerably greater numbers of bacteria after storage in the dry packed product.

In the 1934 tests the products were all packed in paraffin-lined cartons. For comparative purposes two types of containers were used in 1935, sealed cans in addition to the cartons. As far as the survival of organisms during storage in the freezer was concerned, the type of container was without significant effect.

Illustrative of the effect of freezing on the organisms surviving are the average results for bacteria in the 1934 pack, shown in table 10 (see also figure

TABLE 10.—BACTERIA SURVIVING IN FROZEN VEGETABLES AND FRUITS

Average of all methods of packing (1934)	Bacteria per gram					
	Fresh pack	Frozen 6 weeks	Frozen 12 weeks	Frozen 18 weeks	Frozen 24 weeks	Frozen 35-40 wks.
Asparagus	105,000	5,070	4,960	2,880	1,970	1,500
Peas	29,500	4,620	4,790	2,990	2,500	2,500
Beans	51,800	3,630	1,800	930	1,050	1,080
Corn	57,500	6,030	9,330	10,700	8,710	6,370
Strawberries	20,000	9,890	7,410	3,280	2,020	600
Raspberries	50,380	45,700	17,800	1,200	5,820	-

5). Decrease in numbers was more pronounced in the first weeks of storage, after which further decline was much slower, or absent. With the berries studied, decrease in bacteria followed a more uniform rate throughout the experiments. In spite of the decrease noted, however, the studies indicated that even after nine months' storage at 0°F. (—17·8°C.) appreciable numbers of micro-organisms may remain alive on vegetables and fruits. Preservation at this temperature, which is considerably under the critical temperature of 15° to 18°F., below which the growth of micro-organisms may be said to cease, is to be regarded in no way as a process of sterilization.

In the frozen vegetables numbers of yeasts and moulds were relatively insignificant compared with numbers of bacteria. With the fruits examined, however, yeasts and moulds were numerically a more important part of the microflora encountered.

Tests to note the prevalence of organisms of the coli-aerogenes group and anaerobic spores showed that the actual numbers were relatively low. As with total counts, considerable variation was encountered in various batches of freshly packed material, though such differences largely disappeared during storage. Coli-aerogenes types showed a pronounced drop in numbers during the first few

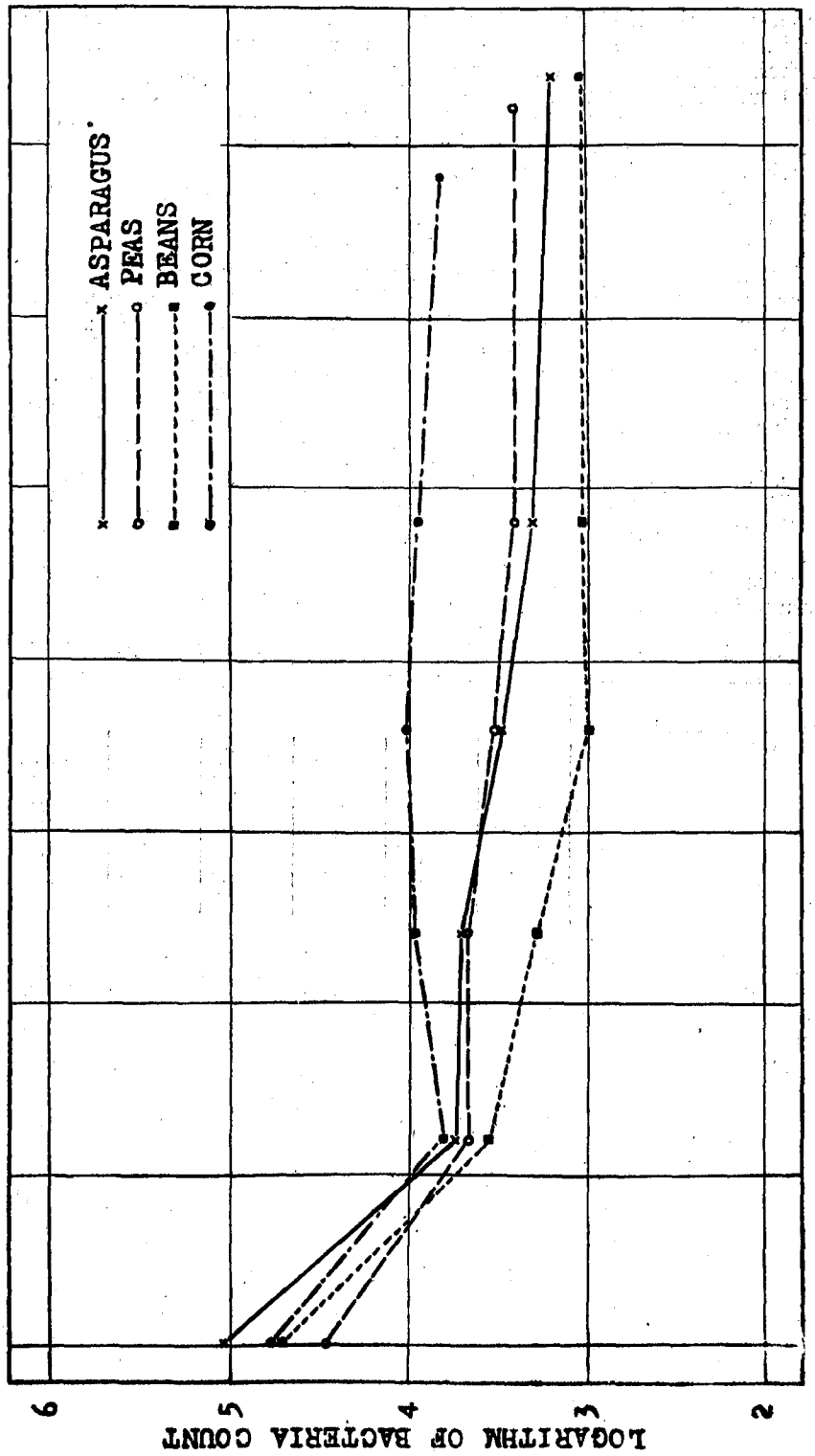

FIG. 5.—Bacteria surviving in frozen vegetables stored at 0° F.

weeks of freezing after which time (six weeks) the numbers were insignificant, particularly with beans and asparagus, the more acid of the vegetables studied and with the distinctly acid berries. As predictable, anaerobic spores, though occurring in small numbers, were more resistant to freezing and persisted in somewhat diminished numbers throughout the period.

Development of Micro-organisms After Defrosting.—To note what development of micro-organisms might be expected by holding frozen vegetables at different household temperatures, tests were made on samples removed from the refrigerator after eight or nine months' storage and allowed to defrost in an electric refrigerator (36°-39°F.), in an ice-box (41°-55°F.) and at room temperature (68°-85°F.).

TABLE 11.—GROWTH OF BACTERIA IN DEFROSTED VEGETABLES

1935 pack, frozen at 0°F. 8 months	Bacteria per gram		
	Electric refrigerator (36-39°F.) 3½ days	Ice box (41-55°F.) 3½ days	Room temperature (65-88°F.) 1½ days
Asparagus	3,180	31,600	12,500,000
Spinach	570	3,500	205,000
Peas	830	5,100	332,000
Corn	4,420	12,160	3,480,000

A portion of the 1935 data is shown in table 11, which illustrates the highly important effect of temperature upon the development after defrosting of bacteria surviving the period of freezing. The electric refrigerator, capable of lower temperatures with less fluctuation than the domestic ice-box, suppresses germ development much more effectively than the latter, while at room temperature enormous bacterial growth and consequent spoilage may be evident within 1½ days. The results emphasize the importance of quick consumption of defrosted products.

Special qualitative tests indicated that increase of coli-aerogenes types was confined chiefly to samples held at room temperature. Tests for anaerobic spores indicated little change while yeasts showed moderate increases depending upon the temperature of holding. With the more acid products, strawberries and raspberries, increases in numbers of micro-organisms studied were less striking than with vegetables.

Types of Bacteria Surviving in Frozen Vegetables.—To note the effect of freezing on the prevalence of various types of bacteria found in freshly packed vegetables, determinations were made of the relative frequency of different generic types present at the start and after approximately nine months' storage at 0°F. (—17·8°C.). The study involved the isolation and examination of all colonies on agar plates or on sectors of plates representative of the products. Similar tests were made from the 1934 and 1935 packs and data secured from a comparative study of 5,197 colonies. The percentage distribution of the various genera in the fresh vegetables and in the frozen products has been detailed elsewhere[1].

Great differences in susceptibility to freezing were noted. *Micrococci* (including *Staphylococci*) and species of *Flavobacterium* were found to survive relatively better than other forms, *Micrococci* in particular comprising noticeably higher proportions of the total numbers after storage than in the fresh

[1]Lochhead, A. G. and Jones, A. H. Food Research, 1: 29-39. 1936 and Food Research, 3: (In press) 1938.

pack (see table 12). *Achromobacter* species, which comprised in most cases a considerable proportion of the bacteria at the start, declined to a marked degree during storage. Organisms of the coli-paratyphoid group, found in varying amounts in the fresh pack, were relatively susceptible to freezing. Contrary to prevailing belief, the proportion of spore-forming bacteria showed no general tendency to increase in the frozen products.

TABLE 12.—PERCENTAGE OF MICROCOCCI IN FRESH AND FROZEN VEGETABLES

Product	Total no. of colonies studied	1934 pack		1935 pack	
		Fresh	Frozen 9 months	Fresh	Frozen 9 months
		per cent	per cent	per cent	per cent
Asparagus	1,123	0·3	33·2	25·5	85·8
Spinach	360	–	–	8·6	63·2
Peas	1,259	18·5	26·0	21·3	44·4
Beans	1,241	5·2	52·9	7·0	72·0
Corn	1,214	11·8	37·7	20·7	78·7

Effect of Freezing on Organisms Developing at Different Temperatures.—To note the effect of freezing on numbers of bacteria capable of developing at various temperatures, counts were made on freshly packed vegetables and on the same products frozen eight months. Comparative tests were made at 4°C. (39·2°F.), 20°C. (68°F.) and 37°C. (98·6°F.).

A summary of the results, given in table 13, shows that maximum counts were obtained at 20°C., indicating that in both fresh and frozen products the

TABLE 13.—EFFECT OF FREEZING ON BACTERIA DEVELOPING AT DIFFERENT TEMPERATURES

1935 pack, frozen at 0°F. 8 months	Bacteria count per gram (averages)		
	4°C. (39·2°F.)	20°C. (68°F.)	37°C. (98·6°F.)
Asparagus—fresh	2,270	4,510	1,970
frozen	6	870	695
% reduction	**99·7**	**80·8**	**64·7**
Spinach—fresh	5,080	10,640	1,680
frozen	185	1,730	695
% reduction	**96·4**	**83·7**	**58·6**
Peas—fresh	880	14,780	6,970
frozen	37	915	495
% reduction	**95·8**	**93·8**	**92·9**
Beans—fresh	1,550	24,330	16,410
frozen	39	1,445	1,060
% reduction	**97·5**	**94·1**	**93·5**
Corn—fresh	71,370	86,210	10,400
frozen	43	11,310	1,610
% reduction	**99·9**	**86·9**	**84·5**

majority of the organisms were types growing best at moderate temperatures. Even after eight months' freezing, surviving bacteria may be regarded as cold-resistant rather than cold-loving. The greatest percentage reduction occurred with bacteria growing at low temperature while those growing at 37°C. were proportionately the most resistant, a finding which appeared surprising with products held in the frozen state. Additional studies provided the explanation

by showing that as the temperature is increased *Micrococci* which had been previously shown to be more resistant than other types, comprise a larger proportion of the colonies developing.

The greater relative survival to freezing shown by *Micrococci,* a group to which consideration must be given in matters of food hygiene, directs attention to their possible importance in relation to the proper handling of frozen vegetables.

Practical Considerations.—The results of the 1934-36 investigations are of practical importance to the frozen pack industry. It has been shown that pronounced decreases in numbers of micro-organisms may occur in frozen vegetable products, particularly during the first weeks of freezing. However, even after months of storage at 0° F. frozen products contain sufficient numbers of living organisms to develop after thawing and cause spoilage if the material is not consumed promptly. Rapidity of spoilage depends chiefly on the temperature.

Freezing at 0° F. is by no means a process of sterilization. Although no microbial growth is possible at this temperature, frozen products are to be regarded as preserved only in so far as they are frozen promptly and kept frozen uninterruptedly. Studies on bacterial growth after defrosting indicate emphatically that re-freezing of defrosted products should not be practised.

Although products frozen in cans are hygienically equal to similar products in cartons, the use of frozen canned goods introduces the possible danger of the consumer's regarding them in the same light as heat-processed products, a psychological factor to be considered.

Frozen promptly, kept frozen continuously until used and consumed promptly, frozen vegetables and fruits are to be regarded as hygienically safe products.

STUDIES OF DETERIORATION OF CHILLED DRESSED POULTRY

In co-operaiton with the Poultry Division, the Live Stock Branch and the National Research Council, studies were undertaken in 1934 and 1935 to investigate the nature of bacterial changes occurring during the storage of dressed poultry held at chill temperatures. Although no growth of micro-organisms is believed possible below –10° C. (14° F.) various species of cold-tolerant organisms have been known to develop at temperatures well below that of freezing. At temperatures approximating 0° C. (32° F.) numerous types of bacteria, moulds and yeasts are capable of growing as shown from studies with organisms from soil, fish, meat, vegetables and other sources. Consequently microbial development might be expected to be an important factor in determining keeping quality of poultry stored at temperature commonly used for the chilled product.

After considerable preliminary experiment to devise satisfactory methods of analysis, an investigation[1] of 144 birds was undertaken. After killing and dressing, the birds were transported to the refrigeration plant of the National Research Council, pre-cooled to 30° F. over night and then packed in the regular manner, eight birds to a box. Two boxes (16 birds) were examined when freshly packed and the remainder stored in equal lots at 30° F. and 32° F. respectively. At the end of two, four, six and eight weeks two boxes (16 birds) were removed from each of the cold storage chambers for bacteriological examination. Bacterial counts were made of the skin and breast tissue of each bird.

[1]For details, see Lochhead, A. G. and Landerkin, G. B. Sci. Agric. 15: 765-770. 1935.

The results from the bacteriological analyses are shown in table 14 in which are summarized the logarithmic averages for the counts obtained from 16 birds in each temperature and storage period group. It will be noted from the table that during the period of storage there is a notable increase in the average bacterial numbers on the skin surface at both temperatures, more pronounced at 32° than at 30° F.

TABLE 14.—SUMMARY OF ANALYSIS OF SKIN AND BREAST MUSCLE OF 144 BIRDS

Temperature of storage	Time in storage	No. of birds	Skin-bacteria per sq. cm.[1]	Breast muscle		
				Bacteria per gr.[1]	pH	Buffer value[2]
30°F.	Start	16	3,380	32	5·70	77·4
	2 weeks	16	20,400	27	5·86	78·2
	4 weeks	16	551,800	121	5·98	77·3
	6 weeks	16	3,635,000	776	5·94	75·5
	8 weeks	16	7,920,000	672	5·99	74·4
32°F.	Start	16	3,380	32	5·70	77·4
	2 weeks	16	30,800	32	5·84	78·7
	4 weeks	16	2,021,000	376	5·92	78·5
	6 weeks	16	7,396,000	1,880	6·01	77·0
	8 weeks	16	25,300,000	290	6·00	75·2

[1]log. average, inner breast muscle.
[2]Increase in millivolts with addition of 25 ml. ·0165 N. HCl after bringing to E.M.F. 135 mv.; inversely proportional to buffer capacity.

It was found that the birds acquired a surface odour before there was any apparent decomposition of, or significant increase of bacteria in, the muscle tissue examined. In addition to the pronounced increase of bacteria on the skin during storage it was observed that the odour from the plate cultures was very similar to that which eventually developed on the stored birds. It is therefore believed that the deterioration of dressed poultry at 30° and 32° F. to the point where the birds acquire a noticeable odour is essentially a surface spoilage due to bacterial growth. With the birds held at 32° F. this initial sign of spoilage was first observed after four weeks, being apparent with those stored at 30° F. at the following (6 wk.) sampling. From fig. 6, in which the logarithms of the average bacterial counts are plotted against time, it is noted that at approximately log. 6·4 (2,500,000 per sq. cm.) the stage is reached at which surface odour is first apparent. From the data there is indication that birds held at 30° F. remain approximately one week longer than at 32° F. before reaching the initial stage of spoilage, five weeks as compared with four.

Compared with the bacterial counts of the skin, those of the breast tissue were very low. During the period of the test increases were relatively slight and irregular, the numbers of organisms being regarded as too low to suggest decomposition. On the other hand, surface deterioration progressed and to the characteristic odour due to bacteria was added, at later stages, the distinctive musty odour arising from the growth of moulds, more particularly on the birds stored at 32° F.

The predominant types of bacteria developing on the skin during storage were representative of the genera *Micrococcus, Flavobacterium* and *Achromobacter,* six species being described, all cold-tolerant rather than cold-loving. Differences in bacterial development at 30° and 32° F. were quantitative rather than qualitative.

A later series of tests was made in which a comparison was made of birds held at 28° and at 30° F. The tests showed that at 28° F. the growth of micro-organisms was definitely slower than at 30° F. Bacterial development was noted at 28° however, although the birds were frozen at this temperature.

Electrometric Tests of Chilled Poultry Muscle.—In addition to the bacteriological examination, electrometric tests were made concurrently of the outer breast muscle of the 144 birds in an attempt to adapt to poultry a method for the detection of the relative freshness of haddock proposed by Stansby and Lemon[1]. The test for freshness of fish is based on buffer capacity measurements and was designed to give information as to the amount of protein breakdown as well as the accumulation of bacterial end products. Differences between poultry and fish meat necessitated modifications in technique. The procedure finally evolved consisted first in titrating with ·0165 N.HCl a definite suspension of ground muscle to an E.M.F. of 135 millivolts with a potentiometer, using the quinhydrone electrode. Buffer capacity was then measured by observing the change in E.M.F. after the addition of definite amounts of acid.

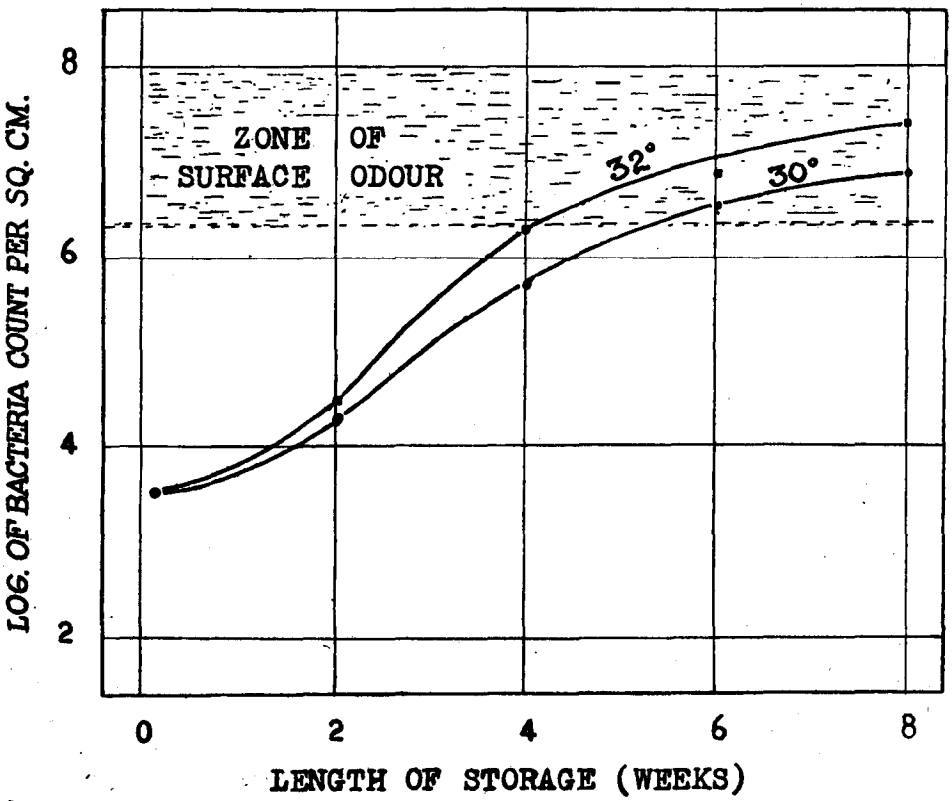

FIG. 6.—Development of bacteria on skin of dressed poultry stored at 30° and 32° F. in relation to surface odour.

The findings, partially summarized in table 14, indicate changes occurring in the flesh of poultry kept at 30° and 32° F. An increase in pH value occurs during the storage period with no significant difference between the two temperature groups. Measurements of buffer capacity, however, showed a preliminary decrease for the first two weeks, followed by an increase which progressed with time. It is of interest to note that in line with the change in buffer capacity,

[1]Stansby, M. E. and Lemon, J. M. Ind. Eng. Chem. (Analyt. Ed.) 5: 208-211. 1933.

amino nitrogen values[1] showed a decrease after two weeks, followed by a significant rise. Similarly Pennington et al[2] reported a decrease in water-soluble nitrogen for the first two weeks at 32° F. (not noticed at higher temperatures), followed by an increase. While no explanation is offered, it is suggested that changes in the proportion of soluble nitrogen compounds account for alterations in buffer capacity observed. As the values observed do no change progressively, but owing to the decrease and subsequent increase are approximately the same after four to six weeks as at the beginning of storage, the electrometric method does not appear applicable as an index of meat condition at 30° to 32° F.

While flesh changes occur immediately on storage at chill temperatures, the bacteriological findings indicate that up to the time the birds develop surface spoilage, such changes are of an autolytic nature, rather than due to decomposition through action of micro-organisms.

Surface Treatment of Dressed Poultry.—To test the possibility of prolonging the period of safe storage of chilled poultry before surface spoilage becomes evident, experiments were made of the effect of treatment by various agents on the development of surface contamination. Included in the tests were several mineral oils, chemical disinfectants (sodium hypochlorite, chloramine-T, formalin and hexyl-resorcinol), brine and wax coating. Co-operation was maintained with the Poultry Division and the National Research Council.

Chlorine disinfectants and brine, used as dips, were ineffective in reducing development of surface bacteria on birds held in storage at chill temperatures. Dipping in formalin showed effectiveness varying with concentration, but the presence of residual formaldehyde detectable after storage made the process unsuited to practice. Hexyl-resorcinol showed some effect with the higher concentration used. Mineral oils, applied with a spray gun, resulted in considerably lower bacterial numbers after storage as compared with untreated birds. Treatment with wax was found to increase considerably the period during which birds remained in good condition.

RELATIVE DECOMPOSITION OF FRESH, FROZEN AND CHILLED POULTRY

The susceptibility of cold stored poultry to decomposition on removal from refrigerated storage, as compared with the freshly killed product, is a matter of considerable importance. On various lots of fresh, chilled and frozen birds, comparative tests were conducted to note the relative degree of decomposition of the meat at different temperatures of holding. The experiments included samples to which mixed cultures of bacteria isolated from spoiled poultry were added, and also samples in which bacterial action was inhibited by addition of toluol, the latter tests being designed to estimate autolytic decomposition.

Bacterial decomposition appeared most active in frozen poultry, while that of chilled poultry meat was intermediate between that of the frozen and the freshly killed product. Purely autolytic decomposition appeared if anything to be most pronounced in the chilled product, less in the frozen birds and least in the freshly killed product. Illustrative of the results is table 15, giving a summary of results in one series. It is suggested that bacterial decomposition in poultry removed from chilled storage is aided by the autolysis during storage, while in the case of birds which have been taken from frozen storage the physico-chemical effects of freezing help to intensify decomposition. Therefore the relative decomposition of chilled and frozen meat may be expected to vary with the time and temperature of storage and the manner of freezing and defrosting.

[1] Determined by the Division of Chemistry.
[2] Pennington, M. E., et al. Proc. Amer. Soc. Biol. Chem., J. Biol. Chem. 29: 31-32. 1917.

TABLE 15.—DECOMPOSITION OF FRESH, CHILLED AND FROZEN POULTRY MEAT

Pre-handling[1]	Temperature of test	Increase in formol titration value					
		Culture added			Toluol added		
		4 days	9 days	13 days	4 days	9 days	13 days
Fresh	28°C. (82·4°F.)	28·7	–	–	1·7	3·1	–
Chilled	"	31·8	–	–	3·0	4·1	–
Frozen	"	42·1	–	–	2·6	3·1	–
Fresh	4°C. (39°F.)	0·0	9·5	21·1	0·3	1·0	1·3
Chilled	"	1·0	6·8	25·1	2·0	2·6	3·5
Frozen	"	0·4	10·9	29·2	1·2	1·1	2·1

[1]Chilled birds held at −1·1°C. (30°F.) for 4½ weeks. Frozen birds held at −22·2°C. (−8°F.) for 4½ weeks.

MICROBIOLOGY OF BACON CURING

The maintenance of an adequate supply of bacon by our packers is a matter of great importance to our export trade and to Canadian agriculture. One problem affecting the success of the bacon industry is that of the condition of the product on the export market. As with other perishable foodstuffs, condition of the product is a matter dependent in a large measure upon means taken to prevent or control the activities of micro-organisms causing changes. Not only are certain definite spoilage defects, such as sliminess, discoloration, etc., but also the less tangible, but all-important question of "quality" dependent upon the proper knowledge and control of microbiological and related biochemical processes in the preparation, curing and storage of the product.

Though micro-organisms are concerned with bacon packing from the time of killing to the end of the storage period of packed sides, relatively little exact knowledge is at hand regarding their activities as affecting cure, spoilage and quality in general. In curing and ripening, bacteria may be at times useful or harmful, though no clear differentiation is yet possible. Though one of the objects of curing, such as practised for Wiltshire sides, is to prolong the life of the meat, it is a process important in affecting appearance, flavour and palatability as well as keeping quality.

Since 1934 studies on bacon curing problems have been carried on by the Division in co-operation with a meat packing plant, chief interest being directed to the organisms in Wiltshire curing pickle. The production of nitrite, which is responsible for giving red colour to cured meat, depends upon bacterial action. Little is known regarding the numbers and types of nitrite-forming bacteria in pickle, their action in causing or preventing storage defects or the effect on them of other pickle bacteria and their mutual reaction to high salt concentration. Studies, still in progress, have been made chiefly on, (*a*) methods for estimating numbers of bacteria in pickle, and (*b*) the types and activities of pickle bacteria.

Quantitative Estimation of Pickle Bacteria.—Comparative tests on a large number of samples have clearly indicated the inadequacy of "standard" methods of analysis for estimating the bacteria in curing pickle. The choice of a suitable incubation temperature and the addition of salt to the medium are important in any quantitative determination. This is illustrated in table 16, showing the average relative counts determined by different methods from a series of pickle analyses. The "standard" method (37° C.) was found to reveal little more than one per cent of the bacteria indicated by the ten per cent salt. The

importance of always maintaining a suitable salt concentration in estimating pickle bacteria was further emphasized in tests showing that exposure to tap water for 45 seconds may kill or render incapable of growth as many as 90 per cent of the countable organisms.

TABLE 16.—EFFECT OF MEDIUM ON BACTERIA COUNTS FROM CURING PICKLE. AVERAGE RESULTS AS RATIOS

Temperature of incubation	37°C.	20°C.			
Salt in medium	0	0	5%	10%	15%
Pickle before filtering	1·3	5·7	52·3	100·0	44·5
Pickle after filtering	1·1	5·1	61·7	100·0	50·3

Qualitative Nature of Pickle Bacteria.—Studies were made of the types of bacteria in pickle and the approximate abundance of different morphological and physiological groups. From representative plates of nutrient agar and 15 per cent salt agar respectively colonies were isolated, to include all on a plate (or a sector) in order to provide quantitative data. From table 17, in which a general

TABLE 17.—BACTERIA FROM CURING PICKLE. SUMMARY OF TYPES AND CHARACTERISTICS

—	Isolated with nutrient agar	Isolated with 15% salt agar
	per cent	per cent
Classification of Bacteria Groups—		
Micrococci—colourless	83·1	9·6
" yellow	5·2	0·0
" orange	0·7	0·0
" pink	2·6	0·0
Rods—non-sporing, Gram pos.	0·7	6·4
" " Gram neg.	1·3	60·9
Rods—pleomorphic—colourless	3·9	23·1
" " pink	2·6	0·0
Physiological characters—		
Nitrate reducing	68·8	91·0
Gelatine liquefying	47·4	5·1
Fat splitting	37·0	8·3
Producing acid from dextrose	58·4	8·3
Growing on nutrient agar	100·0	11·8
Growing on 15% salt agar	22·4	100·0

summary is given, it is seen that depending on the medium used for isolation, the morphological and physiological groupings of the bacteria differ greatly. Whereas micrococci are predominant on nutrient agar, rod forms comprise the most abundant group on media with 15 per cent salt. Apart from the power to reduce nitrates, the salt-tolerant organisms are much less active physiologically than the former group, as judged by their ability to attack protein, hydrolyze fat or ferment sugar.

Based on ability to grow on solid media, organisms in pickle were found to comprise three classes, (*a*) obligate halophiles, for which salt is necessary, (*b*) non-halophilic, unable to grow in the presence of high concentrations of salt and (*c*) facultative, able to grow with or without high concentrations of salt. The presence in pickle of bacteria able to resist, if not multiply in, high concentrations of salt, in addition to others of varying salt requirements for growth, directs attention to the necessity of a thorough investigation of the behaviour of organisms in saline solutions. Proper distinction, not yet recognized, is required between growth, resistance and metabolism. The parts played by the groups indicated are not yet known and further work is being done towards solving this problem.

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 making a study of the sanitary quality of canned tomato juice, pulp, puree, paste, etc. produced in Canada. In 1933 and 1934 extensive preliminary surveys were made, with the object of devising suitable standards for sanitary quality which would permit of a better control of both domestic and imported tomato products.

As a result of the surveys it was felt that the quality of the raw product used and sanitary factory operation could best be estimated by means of a microscopic method of analysis (Howard Mould Count). Accordingly standards for the various types of canned products were set forth, based on the microscopic method, which have permitted a much more satisfactory control of quality in tomato products than formerly possible. Since the establishment of the standards, the Division has continued to co-operate with the Fruit Branch in the microanalytic control of domestic and imported products.

Illustrative of the improvement in quality which has resulted are the figures in table 18, giving the median "Howard Mould Count" of the samples of the various types of domestic products analysed. Better control and increased co-operation with the canneries have resulted in a general lowering of the counts.

TABLE 18.—TOMATO PRODUCTS—MICROANALYTICAL CONTROL

Year	No. of samples examined	Howard Mould Count (Median Count)				
		Juice	Pulp	Puree	Paste	Catsup
1933	76	13	50	66	–	–
1934	221	18	32	50	–	–
1935	821	2	18	24	48	22
1936	900	4	24	20	38	14

ORGANISMS CAUSING GASEOUS SPOILAGE OF CANNED TOMATO PRODUCTS

In connection with the tomato control work on the 1935 pack, a special study was made of samples showing evidence of gaseous spoilage. Of 821 samples of tomato products examined ten were found to be spoiled, six samples of pulp and four of catsup, representing respectively 2·9 per cent and 3·1 per cent of the total number of samples in each group examined. The cans of pulp appeared as typical "swells" while the spoiled catsup likewise showed evidence of abundant gaseous fermentation. Special attention was given to determining the organisms responsible for spoilage, the development of methods for their detection and isolation and to a study of their physiology and the effect of heat in destroying them. A detailed account of the studies is given elsewhere.[1]

The organisms responsible for spoilage were found to be lactobacilli in all cases. From the various samples three species were found as the causal organisms, *Lactobacillus gayoni* in four cases, *Lactobacillus lycopersici* in four cases, and a third species in two cases, closely related to the latter.

For the isolation of lactobacilli causing gaseous spoilage of tomato products, the most satisfactory method consisted in a preliminary cultivation in tomato nutrient broth, followed by surface inoculation of plates of tomato agar (20 per cent tomato juice). This medium, of pH 5·0 to 5·2, was found to be much superior to the more nearly neutral media for the isolation and cultivation of the organisms. For the study of the fermentative reactions of lactobacilli, ten per cent sugar was superior to one per cent, giving more definite reaction.

[1]Jones, A. H. Canadian Canner and Food Manufacturer, 7: 9-14. 1936.

The organisms are readily killed by heat, one minute at 75° C. (167° F.) being sufficient to kill all organisms isolated, even when growing in concentrated tomato pulp. From this it may be concluded that proper heating of the products and care in cleaning and sealing cans and bottles will ensure freedom from losses due to spoilage of this type.

BACTERIOLOGICAL CONTROL OF EDIBLE GELATINE

In recent years the need for establishing some control of the hygienic quality of gelatine entering into food products has become increasingly apparent. Under the auspices of the Department of Pensions and National Health a survey was undertaken in 1935 in which the Division of Bacteriology co-operated with the Laboratory of Hygiene and the Food and Drug Laboratory in the examination of samples of edible gelatine appearing on the Canadian market.

The survey indicated the wide variations occurring in samples from different sources, both as regards total bacterial contamination and in the incidence of organisms of the coli-aerogenes group. As a result of the investigations standards were adopted for the bacteriological control of gelatine, which came into force in 1936. The Division has been co-operating with the Meat and Canned Foods Division through bacteriological control of samples coming within the scope of the Meat and Canned Foods Act. Through the adoption of the bacteriological standards there has been a marked improvement in the hygienic quality of edible gelatine consumed in Canada.

MISCELLANEOUS

THE PRODUCTION OF STERILE BOTTLED CIDER

During the past few years the Division of Horticulture has been developing methods for the production of sterile, self-carbonated bottled cider. Spoilage of an appreciable percentage of bottles led to studies by the Division of Bacteriology in 1934 and 1935 to determine the source of the micro-organisms responsible and suggest remedies.

Efficiency of Seitz Filter.—To test the efficiency of the Seitz filter used to remove micro-organisms previous to bottling, experiments were made at different pressures between 10 and 24 pounds per square inch. In addition to samples taken from the apparatus, cider bottled at each pressure was kept for incubation at 28° C. Storage and cultural tests indicated that sterile filtrates could be obtained at pump pressures up to 24 pounds. This is considerably higher than reported by Carpenter, Pederson and Walsh[1] who regarded 10 pounds per square inch as the maximum safe pressure. Even when the filter was in operation for five hours there was no evidence of micro-organisms "growing through" the filter disks during this time.

Bottle Sterilization, etc.—Attention was next turned to the pressure bottling machine, bottles and crown seals. All of these were found to be sources of contamination of the sterile filtered cider. The type of apparatus for the bottle washing (after preliminary soaking in three per cent caustic solution at 140° F.) was found to be chiefly responsible for the contamination.

Rinsing washed bottles with strong hypochlorite solution (500 p.p.m.), while germicidally effective, was found to cause a certain degree of turbidity and sediment in bottled cider after four months' storage. To avoid this trouble lower concentrations of chlorine in the rinse were employed. In view of the encouraging results obtained in the sterilization of dairy plant equipment with weak acidified solutions (see p. 6), tests were made comparing acidified and non-

[1]Ind. Eng. Chem. 24: 1218. 1932.

acidified rinse at concentrations between 1 and 50 p.p.m., using bottle contaminated with approximately 100,000,000 cells of *Staphylococcus aureus*. After receiving two jet rinses of four seconds duration each, bottles were drained for four minutes and a film of nutrient agar distributed around the inside surface. Bottles were then closed with a sterile crown and incubated at 28° C. for four days.

The first tests showed only a slight difference in favour of the acidified rinse, due to reaction of the latter with the rubber hose connections and resulting loss of strength. When this hose was replaced by block tin tubing and galvanized piping, a somewhat greater difference was noted, but still not enough to warrant adjustment of the pH of the rinse. Both types of hypochlorite rinse were found to be extremely effective in destroying the test organism employed, as the data in table 19 illustrate.

TABLE 19.—EFFICIENCY OF CHLORINE RINSES FOR CIDER BOTTLES

Available Cl. in rinse	Bacterial count per bottle after treatment (log. average)	
	Not acidified (pH=9·0)	Acidified (pH=6·0)
1 p.p.m.	859	221
2 p.p.m.	377	26
4 p.p.m.	27	3·4
10 p.p.m.	2·3	–
20 p.p.m.	1·7	–
40 p.p.m.	less than 1	–

Control rinse with tap water=193,500 bacteria per bottle (average).

ACID PRESERVATION OF ALFALFA SILAGE

In connection with experiments in 1935 by the Field Husbandry Division on the effect of adding mineral acids to alfalfa silage, bacteriological tests were made on the contents of four experimental silos at different stages. The silos contained alfalfa cut in full bloom treated respectively with (*a*) six per cent of 2N hydrochloric acid, (*b*) six per cent of 2N sulphuric acid, (*c*) six per cent of normal hydrochloric and normal sulphuric acid, and (*d*) control without acid.

Throughout the test the control silage remained less acid than the treated lots although in the latter there was a general lowering of the acidity after two weeks so the acidified silages tended to approach the control.

Results of the bacteria counts indicated in the case of the control a normal process for ensiled fodder, showing at first a rapid rise followed by a steady decrease in bacterial numbers. The acid treated lots showed lower bacteria numbers in the early weeks of the experiment, but as time went on numbers rose and after nine weeks all showed higher counts than the control.

Fluctuations between individual samples taken in the earlier stages indicate local variations before the added acid becomes uniformly distributed, and the mass homogeneous. Such variations doubtless aid in the more or less localized development of bacteria and their survival in an environment which would be much less suitable with immediate uniform distribution of acid. Under practical conditions of acid treatment of silage, however, bacteria are by no means rendered inactive, even though they may be definitely suppressed in the earlier stages.

AMERICAN FOULBROOD OF BEES. STUDIES OF BACILLUS LARVAE

New Medium for the Culture of Bacillus Larvae.—In the diagnosis of American foulbrood or in determining the presence of viable spores after treat-

ment of diseased combs, cultural tests for the causal organism, *Bacillus larvae,* form an essential part of the work. As the organism does not develop on ordinary laboratory media, special substrates are required for its cultivation. The need for an effective, simple medium led to studies on growth requirements[1] of *B. larvae* and the development of a medium containing yeast and carrot extract which possessed considerable advantages in effectiveness and simplicity over those formerly used.

In the course of co-operative work with the Bee Division involving the examination of large numbers of samples of treated comb for viable organisms, a new medium containing turnip extract was developed in 1936. It is prepared by adding to 1 litre water, 3 gm. yeast extract, 10 gm. peptone, 0·5 gm. di-potassium hydrogen phosphate (K_2HPO_4) and 200 ml. of clear turnip extract prepared by mincing 100 gm. turnip, grinding with 250 ml. distilled water and filtering. Used either as solid or semi-solid, the medium gives better and more certain growth of *B. larvae* and is now employed for routine examination.

Significance of the Nitrite Test for Bacillus Larvae.—The nitrite test for *Bacillus larvae,* developed by this Division, which depends on the ability of the organism to accumulate nitrite in carrot or turnip media with no added nitrate, is of considerable aid in cultural diagnosis.

Inclusion of this test in the cultural control for viable spores of *B. larvae,* both here and at other laboratories, has confirmed its usefulness. Under similar conditions all other spore-forming bacteria so far tested, as well as many miscellaneous types which may occur in foulbrood diseases or as common contaminants, have not given a similar reaction. To study further the relation of *B. larvae* and other organisms to nitrate reduction and nitrite accumulation and the possible influence of contaminating species in affecting the recognition of *B. larvae,* studies were made in 1936, and reported in detail elsewhere.[1]

TABLE 20.—NITRITE TEST AS AFFECTED BY ASSOCIATION OF *B. LARVAE* AND OTHER ORGANISMS FROM FOULBROOD COMB

Organism	Yeast-peptone-turnip semi-solid medium						
	Inoculated singly		Simultaneous inoculation, *B. larvae* and test organism			Test organism inoculated 48 hours after *B. larvae*	
	Growth	NO_2	Growth of *B. larvae*	Growth of test org.	NO_2	Growth of test org.	NO_2
CONTROLS—							
B. larvae (worker scale)	+	+					
B. larvae (drone larvae)	+	+					
B. larvae (queen cell)	+	+					
TEST ORGANISMS—							
B. orpheus	+	−	fair	good	−	fair	+
B. vulgatus	+	−	fair	good	+	good	+
B. alvei	+	−	slight	good	+	good	+
Bacillus sp.	+	−	fair	fair	+	slight	+
Str. apis	+	−	fair	fair	+	fair	+

[1]Lochhead, A. G. Sci. Agr. 9: 80-89. 1928.
[1]Lochhead, A. G. Can. J. Research, C. 15: 79-86. 1937.

It was shown that whereas *B. larvae* was able to accumulate nitrite in nutrient solutions containing but small amounts of nitrate (0·001 per cent KNO_3), most nitrate reducing organisms showed no accumulation of nitrite at this concentration due to assimilation of nitrate or disappearance of nitrite through reduction or assimilation. With such species, disappearance of nitrite keeps pace with nitrite formation up to a certain concentration, varying with the organism, above which nitrite may accumulate.

To examine the effect of contaminating bacteria on *B. larvae* three strains of the latter isolated from different sources were tested in association with other organisms concerned with brood diseases or occurring as most frequent contaminants on diseased comb. Of five species tested, none showed interference with the accumulation of nitrite by *B. larvae* except *B. orpheus*. With this species a positive nitrite test was dependent on the relative development of the two organisms (see table 20). Further tests showed that *B. larvae* exerted a certain antagonistic action on *B. orpheus*. None of the bacteria grown in association prevented the recognition of growth of *B. larvae* in semi-solid medium by microscopic tests.

COMPARATIVE STUDIES OF SUGAR-TOLERANT YEASTS

Investigations by the Division, previously reported,[1] on the cause of honey fermentation and factors concerning its incidence and control, led to the isolation of numbers of sugar-tolerant yeasts, including various species not previously described. These yeasts, of which species from five genera have been isolated in this laboratory, comprise a group of both theoretical and practical interest, since they are able to grow and cause fermentation in highly concentrated sugar solutions in which "ordinary" yeasts are entirely suppressed.

As time permitted, work has been continued on the classification and physiology of this group of micro-organisms. Previous work[2] had shown the existence in honey of an active principle which stimulated fermentation by certain sugar-tolerant yeasts of the genus *Zygosaccharomyces* in synthetic media. This "bio-activator" could be separated into two fractions and comparative tests with the Toronto strain of *Saccharomyces cerevisiæ* and with Bios I and II of Miller and associates indicated that the stimulation of growth and fermentation was in the nature of a Bios effect.

To compare the response of different yeasts to the stimulating effect of the activating principle, tests were made of the increase in yeast crop and in carbon dioxide production caused by the addition of 10 per cent of honey to a basic salts-sugar solution, sugar concentration being maintained at 40 per cent throughout. The results, based on measurements under uniform experimental conditions, are shown in figure 7 which indicates the pronounced effect of the bio-activator in causing increase in yeast growth and in fermentation. While individual species showed different responses, the *Zygoscaccharomyces* as a group reacted more intensely than members of other genera tested, with the *Torula* species showing the least response. The results are of much interest in connection with previous studies of honey spoilage under practical conditions, which showed that the chief agents of fermentation were members of the genus *Zygosaccharomyces*.

GENERAL ANALYTICAL SERVICE

In continuance of the policy maintained in previous years, assistance was given, particularly to the farming community, but also to other Divisions and Branches of the Department, through the examination of samples submitted for bacteriological analysis. During the three-year period 1934-36, the total

[1] Reports of the Dominion Agricultural Bacteriologist for 1927-28, for 1929-30 and for 1931-33.
[2] Lochhead, A. G. and Farrell, Leone. Can. J. Research, 5: 529-538, 539-543. 1931.

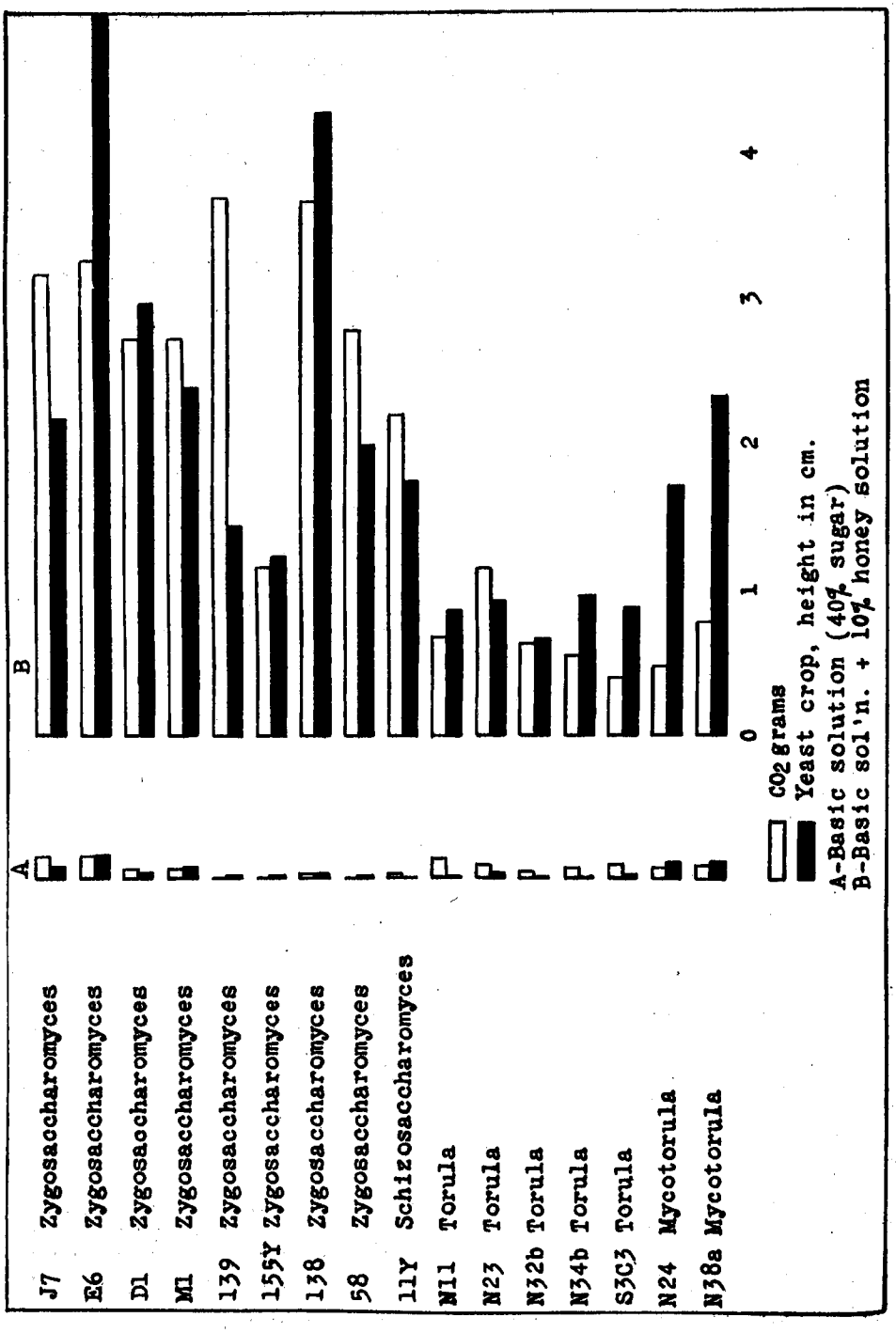

FIG. 7.—Effect of bioactivator in honey in stimulating growth and fermentation of different species of sugar-tolerant yeasts.

number of samples received was 4,711 exclusive of routine milk samples from the Experimental Farm dairy. The samples submitted 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 it is but to be expected that the analytical work involved varied greatly. While some samples required but a routine testing others necessitated a much more extended investigation.

During the period 343 samples of well water were received for analysis, the findings being briefly summarized in table 21. In general, results of the analyses have corresponded to those of former years and indicate that with many farm wells location and construction leave much to be desired. In a large proportion of cases the source of supply is the shallow, dug well which offers greater chance for contamination than the drilled or driven wells which penetrate to lower sources of water. Apart from the question of depth, it is important that

TABLE 21.—ANALYSES OF FARM WELL WATERS

	1934	1935	1936
Number of samples analysed	111	112	120
Per cent free from pollution	29·8	27·7	33·3
Per cent polluted	28·8	29·7	24·2
Per cent of doubtful quality	41·4	42·6	42·5

the well be located at a safe distance from such sources of pollution as stable, manure pile, privy, cesspool, etc., with due consideration to ground flow. Furthermore, the analyses indicate that in many wells surface contamination is reaching the water due to faulty construction of well-covers and cribwork, permitting the entry of bacteria which would be otherwise kept out by the filtering action of the soil.

A wholesome water supply should be the aim of every farmer. In having a test made it is important that examination be made as promptly as possible after taking the sample. It is therefore recommended that tests be made at the nearest laboratory. This Division is glad to make bacteriological water analyses. It is advisable to make application first in order that directions for taking the sample may be given.

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