ON ROPE (AND SOURNESS) IN BREAD.

TOGETHER WITH A METHOD OF ESTIMATING HEAT-RESISTANT SPORES IN FLOUR.

By D. JORDAN LLOYD, A. B. CLARK and E. D. McCREA.

(*From the Biochemical Laboratory, Cambridge.*)

CONTENTS.

1. Introduction.

During the summer of 1917, we were asked by the Food (War) Committee of the Royal Society to investigate the factors responsible for the outbreak of rope in the nation's bread which started early in June and rapidly assumed serious proportions. The following notes form a summary of two reports presented by us to the Committee, and of some further investigations undertaken on behalf of the Wholesale Co-operative Society. We wish to thank both the Royal Society and Dr Geoffrey Martin of the Wholesale Co-operative Society for permission to republish our results.

The earliest reference to the condition now known as rope occurs in a paper by Laurent (1884). He stated that "pain visqueux" was of common occurrence during the summer months amongst the agricultural population of Belgium accustomed to bake their own bread. He ascribed the condition ("pain malade" or "visqueux") to a bacillus which he isolated from the bread and named *B. panificans*. His description of "pain visqueux" is sufficiently striking to be given verbatim: "Pendant les mois les plus chauds de l'année, de juin à septembre, il arrive souvent que le pain préparé dans les ménages de la campagne subisse des transformations d'une nature toute spéciale. Deux ou trois jours après le cuisson il répand une odeur putride; consommé alors, il a un goût sucré qui ne déplaît pas. Peu de temps après, l'odeur devient plus forte, et ne tarde pas à rappeler celle des matières

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albuminoides en décomposition. Un couteau introduit dans la mie se couvre d'une matière gluante qui se détache difficilement. Si l'on enfonce le doigt dans la partie centrale de la mie et que l'on le retire lentement, il entraîne des lambeaux qui prennent la forme de filaments analogues à ceux que donne la colle forte."

The cultural characters of Laurent's *B. panificans* are given below. He isolated this organism from ropy bread and also from flour, grains of wheat, and soil. He considered that the organism was universally present on the surface of cereals and passed into the flour during milling. He stated that it could resist the heat of baking, and develop in bread, attacking both gluten and starch. He realised that the activity of the bacillus was dependent on both the moisture and the reaction of the bread, and asserted that "pain visqueux" could be prevented by the addition of vinegar to the dough.

A number of later workers substantially confirm Laurent's conclusions. Kratschmer and Niemilowicz (1889) and Watkin (1906) considered that *B. mesentericus vulgatus* (Flügge) is a causative organism, and Watkin also attributed rope to *B. mes. fuscus*. Uffelmann (1890) considered that two types of bacilli called by him *B. liodermos* and *B. mes. vulgatus* (Flügge) could both cause rope in bread. Kries (1893) also attributes rope to a bacillus unspecified. Vogel (1897) made a detailed investigation of rope during an outbreak in Hamburg. He isolated three strains of bacilli of the *Mesentericus* group from affected bread, and considered that two of them, called by him *B. mes. panis viscosus* I and II respectively, were capable of causing rope in bread. Both types produce a highly alkaline reaction in affected bread. Type I is stated to have a proteolytic action on gluten but no action on starch, type II to hydrolise both gluten and starch. Beattie and Lewis (1917) consider that only one species of bacillus (characters not given) can cause rope. They state that the condition of rope is to be attributed to a "viscous fermentation of carbohydrates." They call their organism *B. mes. panis viscosus* (Vogel), but it is to be noted that Vogel states that his commoner type has no action on starch.

All previous workers on rope agree in attributing the condition to a bacillus of the *Mesentericus* group. The cultural characters of the bacilli isolated by them are given in Table I. below. They state that the spores of the bacilli occur on cereals and resist baking. Most workers state that ropy bread is alkaline in reaction and that the condition can be inhibited by the addition of acid to the dough. Several emphasise the importance of moisture and temperature. From their descriptions it is not easy to differentiate between the conditions found in bread in different outbreaks, but it is evident that the bread was not affected in the same way in the various outbreaks which have been investigated. Our own investigations made during the summer of 1917 have led to the isolation from affected bread of five different strains of bacilli all belonging to the *Mesentericus* group, each of which in pure culture is capable of causing the decomposition of bread. These and other bacilli of the *Mesentericus* group can also be isolated from flour, from the outside surface

of grains, wheat, barley, maize, etc., from the dust in mills and bakehouses, and from sound bread. There seems little doubt that they are normally present in all breads, but their presence does not give rise to rope unless conditions prevailing are such as to allow of great multiplication. Under such conditions the changes they produce are:

(1) an unpleasant smell,

(2) a brown discolouration,

(3) a sticky disintegration of the crumb due to the hydrolysis of either the bread proteins or carbohydrates or of both,

(4) a change in reaction which may become markedly alkaline, markedly acid, or neutral. (Normal unaffected bread has a faintly acid reaction.)

In the alkaline conditions the characteristic stringiness so graphically described by Laurent, develops very rapidly. In the acid or "sour" breads it does not usually occur until decomposition is more advanced. In their early stages of development, it is impossible, except by a chemical and bacteriological examination, to distinguish between the decomposition caused by the different types of infection.

2. Isolation of Causative Organisms from Bread, Flour and Grains.

Affected loaves were examined by the usual aerobic methods, strains of bacteria isolated from the loaves being obtained in pure culture, and re-inoculated into sterile bread to test their action. Five types of bacilli were isolated from affected bread, all belonging to Flügge's *Mesentericus* group. These are referred to as A, B, C, D and E. The loaves were further examined by the methods of anaerobic culture, glucose broth and glucose agar being used. No obligatory anaerobes were isolated by us in either a hydrogen or a carbon dioxide atmosphere. Type A proved to be a facultative anaerobe in both hydrogen and carbon dioxide, types B to E showed slight surface growth in the carbon dioxide atmosphere, none at all in hydrogen. Types A and B were the types encountered most often, C and D were only encountered once each, E three times out of a total of 20 loaves examined.

Whole grains of wheat, barley and maize were examined. Since the only organisms of importance for our investigation were those capable of forming heat-resistant spores, the grains were put into sterile broth or water, and steamed for 20 minutes to destroy non-spore bearing forms. Fifty different samples of grains from every part of the world were examined, every one of which proved to be carrying heat-resistant spores capable of causing unsoundness in bread. Four grain samples from the scourers were also examined and found to be carrying rope bacilli. B was the commonest type, being isolated from 60 per cent. of the grain samples examined; A and E are common; C rather more rare, D was not isolated from any grain sample, but an additional type N was encountered once on a sample of barley. This type N appears to be identical with *B. mesentericus niger* originally isolated by Biel (1896) from bread.

Over 100 samples of flour were examined. 0·5 gram of flour was steamed for 20 minutes in 10 c.c. of sterile water, and the emulsion was inoculated on to agar plates. B, A, E and C are again the types usually found. Type N was encountered twice.

3. Cultural Characters of the Organisms.

The cultural characters of the strains of *B. mesentericus* A–E and N were examined by growth on various media. Cole and Onslow's tryptic broth was used as the basis for all nutrient media, and the reaction was adjusted to be neutral to phenolsulphone phthalein. ($P_H = 7·3$ on Sørensen's logarithmic scale (1909)). Gelatine media contained 10 per cent. of gelatine, and agar media 2 per cent. of agar.

A few special media were employed. Bread plates were made by cutting small slices of bread, a quarter of an inch thick, and sterilising them in Petri dishes in the autoclave for 20 minutes at 115° C. In all experiments with bread plates, control bread plates moistened with sterile water were incubated with the inoculated plates for comparison. Gluten plates were made by mixing gluten flour with tap water in such proportions that a thick, but not sticky, paste resulted. The paste was placed in Petri dishes and autoclaved. During sterilisation the gluten paste "rises" somewhat and becomes of a much firmer consistency. A starch medium was made for testing the action of the bacteria on starch. This was made by adding seven grams of pure cornflour starch to 100 c.c. of neutral broth with phenol red as indicator. The starch suspension was well shaken, tubed, gelatinised by boiling, the tubes being well shaken until gelatinisation was complete, and sterilised in the autoclave. This medium was used for stab cultures. The cultural characters of our types A–E and N are all summarised in Table I, together with the characters of the organisms described by previous workers. The most striking features are the very slight proteolytic power of A, and the very marked proteolytic power of B, C, D, E. It can be seen that the organisms cause a series of decompositions in bread passing from acid to alkaline in the final reactions. Fermentation tests with strains A–E were made with glucose, lactose, saccharose, mannite, and salicin. The results were not specific. The heat-resistant power of the spores of all five organisms is a very important economic character. Pure cultures of types A–E were sealed into five capillary tubes and embedded in dough and baked. The time of baking was three-quarters of an hour. In every case the original organism was subsequently recovered. Vogel's experiments on the influence of the heat of baking had similar results. Experiments with broth cultures at 100° C. showed that the survival times of the various species differed considerably. Values are given in the table.

Another important cultural character is the inhibition of growth by acid. Growth in all strains is inhibited by a reaction of $P_H = 5·5$, a figure which strangely enough coincides with Jesson-Hansen's optimum reaction for gluten. This same limiting value for the growth of rope bacilli was also reached by

Table I.

	B. panificans (Laurent)	*B. liodermos* (Flügge)	*B. mes. vulgatus* (Flügge)	*B. mes. fuscus* (Flügge)	*B. mes. panis viscosus* I (Vogel)	*B. mes. panis viscosus* II (Vogel)
Form	Bacilli, short rods	Bacilli	Bacilli	Bacilli	Bacilli, thick rods	Bacilli, slender rods
Motility	+ve	—	—	—	−ve	+ve
Gram	—	—	+ve	+ve	+ve	+ve
Spores	Central	—	—	—	Central	Position not described
Powers of heat resistance	Resist baking (interior of loaf never above 100° C.)	—	—	—	Survives 100° C. for 1 hour	Resists 100° C. for 15 minutes
Anaerobic culture ...	+ve in an atmosphere less than 1 mm. Hg	+ve	+ve	+ve	−ve	+ve (slower growth than in air)
Agar slope	—	White rosette-like growth	Grey-white glistening and crumpled growth	—	Grey-brown granular growth, with outgrowths into the medium	Grey-white, dry and crumpled growth
Broth	—	—	Weakly turbid, with a tough membrane	—	Weakly turbid No change in reaction	Clear, with a tough membrane Faintly alkaline
Potato	—	Gummy, translucent growth	White or pale yellow crumpled growth	Yellow-grey, moist and crumpled growth	White-grey growth, at first slimy, later crumpled	White-grey growth, at first slimy, later crumpled
Gelatine stab	Liquefaction on the surface and leaf-like growth	Funnel liquefaction with membrane formation	Funnel liquefaction, becoming tubular, with membrane formation	Slow funnel liquefaction with membrane formation	Slow funnel liquefaction	Rapid funnel liquefaction
Milk	—	Coagulated, peptonised. Butyric acid produced	Slow coagulation and peptonisation Reaction. Alkaline	Coagulated, peptonised. Undergoes a "slimy fermentation" Reaction. Alkaline	Precipitation of the casein with slow solution	Precipitation of the casein with slow solution
Bread	Ropy	Ropy	Ropy	Ropy. Becomes brown, viscous, and evil-smelling	Ropy Reaction. Alkaline	Ropy Reaction. Alkaline
Gluten	—	—	—	—	Liquefaction	Liquefaction
Starch	—	—	—	—	No change	Rapid liquefaction
Optimum P_H	—	—	—	—	—	—
Limiting P_H	—	—	—	—	—	—
Optimum temperature ...	33° C.	—	—	—	35°–37° C.	40°–42° C.

	Type A	Type B	Type C	Type D	Type E	Type N
Form	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli
Motility	+ve	+ve	+ve	+ve	+ve	+ve
Gram	+ve	+ve	+ve	+ve	+ve	+ve
Spores	Central	Central	Central	Central	Central	Central
Powers of heat resistance	Resists 100° C. for 3 hours	Resists 100° C. for 6 hours	Resists 100° C. for 30 minutes	Resists 100° C. for 6 hours	Resists 100° C. for 1 hour	—
Anaerobic culture in H_2	+ve	−ve	−ve	−ve	−ve	—
Anaerobic culture in CO_2	+ve	+ve	+ve	+ve	+ve	—
Agar slope	Grey - white, crumpled and moist growth, with a distinctive bullous appearance closely adherent	Grey - white, moist, opaque and crumpled growth	At first a slimy, white growth, later becoming crumpled	Moist, grey - white growth, punctate appearance, never crumpled (poor growth)	Dry, grey-white, finely crumpled growth	White wrinkled growth, medium stained black
Broth	Turbid, with a tough membrane, bullous in appearance, and of a pinkish colour Alkaline	At first turbid, later clear, with a tough, crumpled membrane Alkaline	Turbid, with a thin membrane Alkaline	Slowly becomes turbid, with a slow formation of a thin film Alkaline	Slight turbidity, with a tough, crumpled membrane Alkaline	—
Potato	Pink - brown, finely plicated growth, bullous appearance, adherent	White-yellow, crumpled growth Potato blackened	Slimy white growth, later crumpled	White crumpled growth	Green - yellow, finely crumpled growth	—
Gelatine stab	Extremely slow liquefaction	Rapid funnel liquefaction	Liquefaction	Slow liquefaction	Liquefaction	—
Milk	Coagulated Acid	Precipitation of the casein with peptonisation Alkaline	Precipitation of the casein with peptonisation Alkaline	Precipitation of the casein with peptonisation Alkaline	Precipitation of the casein with peptonisation Alkaline	
Bread	Pinkish - brown stain, softened, sticky and crumbly Neutral	Yellow to dark brown stain, consistence resembling that of honey Alkaline	Green - yellow stain, softened, sticky and crumbly Acid	Yellow - brown stain, consistence as B Alkaline	Green - yellow stain, softened, sticky and crumbly Acid	Inky stain, softened Acid
Gluten	Surface growth. Not noticeably softened NH_3 given off. Alkaline	Surface growth. Gluten becomes a slimy, semi-liquid mass NH_3 given off. Alkaline	Surface growth. Gluten much softened NH_3 given off. Alkaline	Surface growth. Gluten becomes slimy and semi-liquid NH_3 given off. Alkaline	Surface growth. Gluten much softened NH_3 given off. Alkaline	—
Starch	Slow liquefaction. Pellicle formed Alkaline, then acid	Rapid liquefaction Acid	Rapid liquefaction Alkaline	Rapid liquefaction Acid	Liquefaction Reaction unchanged. P_H 7·4	—
Optimum P_H	6·5	7·5	6·5	7·0	6·5	—
Limiting P_H	5·5	5·5	5·5	6·0	5·5	—
Optimum temperature ...	About 37° C.	About 37° C.	About 37° C.	About 37° C.	About 37° C.	—

Cohn in some subsequent work on rope in America. The work is reported by Henderson (1918).

4. Chemical Changes produced in Bread by the Bacilli.

These fall readily under three headings:

(1) Changes in reaction.
(2) Changes affecting the proteins.
(3) Changes affecting the carbohydrates.

Changes in reaction were tested by inoculating sterile bread plates with pure cultures, incubating for 48 hours at 37° C. and testing with indicators, and comparing the colour with that of a sterile control bread plate. The error is about ± 0·25 on the scale of units used. The results are given below.

Table II. *48-hour bread cultures.*

Bacillus	Consistency of bread plate	Colour of bread plate	Reaction of bread plate	Buiret test on watery extract
Control	Normal	Normal	Neutral to brom-cresol purple. $P_H = 6·5$	Negative
A	Sticky, softened	Pink, later brown	Acid to phenol red, alkaline to brom-cresol purple. $P_H = 7·0$	Negative
B	Very viscous, semi-liquid	Brown	Alkaline to phenol red and litmus. Neutral to cresol red. $P_H = 8·0$	Pink colour
C	Sticky, softened	Greenish-yellow, later brown	Acid to phenol red, litmus, brom-cresol purple. Neutral to methyl red. $P_H = 5·5$	Mauve colour
D	Very viscous, semi-liquid	Yellow, later brown	Alkaline to all indicators used. $P_H = 9·0$ (circa)	Pink colour
E	Sticky, softened	Yellow, later brown	Acid to litmus, phenol red and brom-cresol purple. $P_H = 5·5$	Mauve colour

There seems to be a close relation between the reaction produced by the infecting bacillus and the destruction of the bread proteins. Type A which produces a very slight shift of the reaction towards the alkaline side, has very little proteolytic power—too little to detect in a 48-hour bread culture; grown on gluten, the gluten plate becomes covered with a pink encrusted growth, there is a smell of ammonia, showing that the protein is de-aminated but the gluten remains firm and consistent, and no water soluble bodies are produced that give the Biuret reaction. B and D which produce alkali on bread and on gluten, rapidly liquefy both media. The advanced stage of proteolysis is shown by applying the Biuret test to watery extracts of bread and of gluten. In both cases a deep rose-pink colour is produced. In bread infected with B, it is readily shown by a formol titration, that not only albumoses and peptones, but also large quantities of free amino-acids are produced.

A and C which produce an acid reaction in bread, have a less violent proteolytic action, possibly because the acid produced is sufficient to bring the reaction near to a value which inhibits growth.

Laurent and Vogel both find that protein break-down occurs in breads infected with the bacilli isolated by them.

Cultures of the bacilli on a gluten medium, however, show a series of protein liquefactions exactly parallel to the series on bread, though in every case on gluten the medium becomes alkaline and there is evolution of free ammonia.

This difference of reaction produced on bread and on gluten by the five types under consideration is of considerable chemical interest. The reaction in the bread cannot be explained simply as the sum of the reactions of the various organisms on the protein and carbohydrate respectively, since though A, B, D and E all produce an acid reaction in starch stabs, C, which is an acid producer on breads, produces alkali not only from gluten but also from starch.

Normal bread contains starch, dextrins and reducing sugar. Laurent states that his *B. panificans* attacks cooked starch with the production of an erythrodextrin. Vogel stated that *B. mes. panis viscosus* II had a strong diastatic action and that in an infected loaf there was an accumulation of reducing sugars, mainly maltose and glucose. We have found that in bread infected with our type B there is an increase of a reducing sugar identified as glucose. The quantity of reducing sugar in the loaf may be used as an index of the progress of the decomposition. Hüppe (1884) has shown that *B. mesentericus* as a group is characterised by possessing a diastatic enzyme and Effront (1917) considers that this enzyme is an acrodextrinase.

5. Method of Estimation of Heat-resistant Spores in Flour, and Distribution of the Spores in Flour-mills and Bakehouses.

The method described below for estimating the degree of infection of flour with deleterious bacteria has been published in the reports of the Food (War) Committee of the Royal Society (Jordan Lloyd and McCrea, 1917). The figures for infection of flours given in this report were all obtained under war conditions, but we feel that it would be of more general value to give here results obtained under a more normal state of affairs. We have, further, standardised our method of examining flour; a detailed description is given below:

(1) 300 c.c. of distilled water is placed in a 500 c.c. flask. This is plugged with cotton wool and autoclaved.

(2) 100 g. of the flour is weighed out and shaken into the sterile water with constant stirring.

(3) The flask containing the flour and water is well shaken.

(4) Tubes containing 10–20 c.c. of nutrient agar are melted, four of these being allowed to each sample of flour.

(5) 4 c.c. of the flour emulsion is removed from the flask with a sterile graduated pipette, 1 c.c. being placed in each of the four tubes of melted agar.

(6) The tubes are placed in boiling water for 20 minutes.

(7) The contents of each inoculated tube are poured into a sterile Petri dish.

The colonies of *Bacillus mesentericus* are counted after 24 hours' incubation at 37° C.

The flour is then judged by the total number of colonies on the four dishes.

If there are 12 (or less than 12) colonies, the flour is to be considered sound.

If there are between 12 and 50 colonies, the flour is liable to develop rope under bad conditions.

If there are more than 50 colonies, the flour is to be regarded as unsafe for ordinary commercial use.

Experimental results are given below. They show the low degree of infection in normal flour, the order of infection found where rope has occurred, and the stages in the milling process at which the rope spores are liable to accumulate.

Case 1. *Samples collected from a flour-mill.*

Number of sample	Description	Total number of colonies on the four plates	Dominant type of *B. mesentericus* (1)
1	Wheat berry ground up	14	A, B and C
2	Break flour	22	A, B and C
3	"Dead" flour from break rollers	∞	A, B, C, E, etc.
4	"Dead" flour from ledges and "sweat" from break middling machine	∞	A, B, C (and maggots!)
5	Crude semolina	2	—
6	Pure semolina	1	—
7	"Dead" flour from ledges of semolina machine	17	A and C
8	Finished flour from sack	7	A
9	Finished flour from different sack	5	A
10	Bran	28	A, B and C

Case 2. *A further series of samples from the same flour-mill as Case* 1.

Number of sample	Description	Total number of colonies on the four plates
1	Manitoba wheat from silo S. 9	2
2	Manitoba wheat going on drier from whizzer ...	0
3	Manitoba wheat leaving drier	3
4	River Plate wheat from silo S. 10	8
5	Plate wheat going on drier from whizzer	0
6	Plate wheat leaving drier	0
7	English wheat from finch	2
8	English wheat going on drier from whizzer ...	2
9	English wheat leaving drier	5
10	Australian wheat going on drier from whizzer ...	1
11	Australian wheat leaving drier	4
12	American winter wheat from silo M. 3	8
13	Clean winter wheat after standing 12 hours ...	4
14	American maize nuts	36
15	Aspirations from wheat separator	16
16	Mixed wheat going on mill after standing 12 hours	4
17	1st break	2
18	2nd break	2
19	"Dead" flour from second break rollers	4
20	Reduction semolina A	1
21	Reduction semolina B	12
22	Reduction semolina C	4
23	Finished flour	18

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Case 3. *Samples collected from a different flour-mill.*

Number of sample	Description	Total number of colonies on the four plates
1	Winter wheat going on drier from whizzer	0
2	Winter wheat leaving drier	0
3	Grind 1st break	3
4	Grind 2nd break	3
5	Break flour	1
6	Fine middlings	2
7	Semolina	0
8	Finished flour	0

Case 4. *Samples of flour taken from a bakery where rope had occurred in the bread.*

Number of sample	Description	Total number of colonies on the four plates	Dominant type of *B. mesentericus*
1	Flour scrapings from floor and ledges in flour stores	610	A 30 % B 60 %
2	Flour in bag attached to automatic mixer	130	A 50 % (and numerous B 50 % small beetles)
3	Flour adhering to canvas shoot from hopper	30	A 50 % B 50 %
4	Brushings from outside ledges of a trough	2000 (about)	A 30 % B 30 % *B. subtilis* 30 %
5	Flour I	342	A
6	Flour II	1	—
7	Flour III	5	—
8	Flour IV	5	—
9	Flour V	3	—
10	Flour VI	4	—
11	Flour VII	7	—
12	Flour VIII	7	—

In case 4, out of eight flours examined, seven were "sound" and one was found to be heavily infected with *B. mesentericus* type A. The bread, however, on examination showed that the rope was the alkaline type characteristic of *B. mesentericus* type B. The source of infection, therefore, was not primarily to be traced to flour I but to the dirty condition of the bakery, as shown by samples 1–4.

Case 5. *A second series of samples from the same bakery as case* 4.

Number of sample	Description	Total number of colonies on the four plates	Dominant type of *B. mesentericus*
1	Flour scrapings from ledges in flour store	800	A, B and C, and numerous others not identified
2	Brushings from the outside ledges of a trough	145	As above
3	Dough from flour of mixing room	1000	A, B, E, etc.
4	Flour I (sample milled a week later than in case 2)	114	A
5	Flour I (sample milled 3 days later still)	11	A

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These examples show very clearly how an infected mill will clean itself out during subsequent millings, the three samples of flour I showing degrees of infection 342, 114 and 11, all being milled within ten days of each other.

6. Conditions Controlling the Development of Rope.

We have shown that spores of the *Mesentericus* group are universally present in flour and bread, but their presence is a matter of no importance unless conditions are such as to favour great development. The five principal factors concerned in such development are:

(i) Degree of infection.
(ii) Moisture.
(iii) Temperature.
(iv) Reaction of the bread.
(v) Composition of the flour.

(i) *Degree of infection.* Watkin (1906) and Semler (1917) give instances of occurrence of rope attributable to highly infected batches of flour; Beattie and Lewis give an instance where indirect evidence suggested that an increased degree of infection was the cause of an outbreak. We have had the opportunity of examining the flour connected with a serious outbreak of rope in a military camp, and found it very highly infected. In another case where rope developed in two loaves from a certain baker's shop, the flour was not infected to a greater degree than the normal war-flour of the year 1917. The bakery is a very reputable one, and both loaves came from a batch of bread baked on a Friday and kept over a week-end during a spell of very hot weather in June. In the instance of rope in a bakery analysed in a previous section, it was shown that though an infected flour was concerned in the outbreak, the main source of trouble was the contamination of the dough in the bakehouse. This case is a good example of how infection in bread may come from both internal and external sources.

(ii) *Moisture.* The importance of the moisture content of a loaf was stated by Vogel and is indeed clearly recognised in the practice of modern large-scale bakeries where the freshly drawn bread is placed on the floor of the store house which is arranged with a through draft to draw off the steam rising from the warm loaves. In the early days of the enforcement of the 24 hour bread order, the freshly baked bread was frequently stacked while hot and steaming in the delivery carts, or some other closed space in order to prevent loss of water and to maintain the weight of the loaf. This habit as may be imagined led to a considerable number of cases of rope. The following experiment shows the influence of the moisture content of the loaf on its capacity to develop rope. A dough was made in the usual manner and divided into portions each weighing one pound. After proving, the doughs were all baked together in a slow oven at 125° C. They were withdrawn at different times. Loaf III may be considered a "normal" loaf, loaves I and II were under-

baked, loaves V and VI over-baked. After baking and cooling the loaves were cut in two, and the control halves were estimated for reducing sugars. The other halves were incubated for 48 hours in a moist incubator at 37° C. All developed rope of the B type. The reducing sugars were then estimated in the ropy halves. In all loaves the method of estimation was the same. Fifty grams of bread from the middle of the loaf were extracted three or four times with boiling water. The total volume of the extracts was made up to 250 c.c. Each extract was mixed with 500 c.c. of alcohol to precipitate the dextrins, allowed to stand 24 hours and filtered. The alcohol was removed by distillation *in vacuo* and the total again made up to a known volume. 125 c.c. was found to be a convenient volume for extracts from unincubated, and 250 c.c. for extracts from incubated loaves. The results are given in the table below. The sugar was estimated by Benedict's method. The amount of sugar present is expressed as a percentage of the weight of the uncooked dough.

Table III.

Loaf	Time of baking, mins.	Weight, oz.	Sugar in control half, %	Sugar in ropy loaf, %
I	30	15·5	1·1	6·38
II	45	14·5	1·0	11·8
III	60	13·75	·9	9·5
IV	75	12·9	1·0	7·23
V	90	12·25	·9	4·73

This experiment illustrates two points: (1) that *B. mesentericus* does not readily attack uncooked starch, (2) the importance of the moisture content.

(iii) *Temperature.* Rope is essentially a warm weather trouble as is recorded by Laurent, Vogel, Watkin, etc. The outbreak with which we were concerned was at its height in June, but cases continued to occur throughout the summer.

(iv) *Reaction.* The reaction of the growth medium is well recognised as having considerable influence on the rate of growth of micro-organisms, and acid breads have been found by all investigators of rope to be immune from the condition. An experiment was made by adjusting nutrient broths to varying reactions, to investigate the limits of growth of *B. mesentericus* types A–E. The broths were adjusted according to the instructions in Cole and Onslow's paper (1916). The results are given in the table below. 0 signifies no growth; + very slight growth; + = slight growth; + + = good growth; + + + = very heavy growth. The results are taken from 36-hour cultures.

Table IV.

Reaction of broth	*Bacillus mesentericus* type A	B	C	D	E
P_H = 8·0	(+)	(+)	(+)	(+)	(+)
7·5	+	+ +	+	+ +	+
7·0	+ +	+ + +	+ +	+ + +	+ +
6·5	+ + +	+ + +	+ + +	+ +	+ + +
6·0	+ +	+ +	+ +	0	+ +
5·5	0	0	0	0	0
5·0	0	0	0	0	0

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It can be seen that the optimum reaction for growth is $P_H = 6{\cdot}5$. This unfortunately coincides with the reaction of normal bread. The inhibitory reaction $P_H = 5{\cdot}5$ coincides with Jesson-Hansen's value for the reaction to be desired in a dough in order to attain the optimum condition of the gluten. It is, therefore, from both points of view the theoretical ideal for bread. Its attainment in bakehouse practice, however, presents difficulties.

(v) *Composition of the flour.* There is a very definite belief in the baking trade that brown bread is more liable to rope than white bread, and much circumstantial evidence tending to confirm this. This difference might be due to a varying in the degree of infection, the natural supposition being that brown flour containing branny particles would have a larger number of bacteria than white flour from the middle of the wheat berry, or it might be due to the brown bread being a more stimulating medium of growth. Both these possibilities were examined. Only one sample of wholemeal flour was available at the time the examinations were made. It certainly had a high degree of infection, 180 colonies developing on the agar plate compared with values varying from 30 to 70 obtained from flour of 70, 80 and 90 % extraction. The differences among the latter were not sufficiently striking to have been due to anything but chance. The possible influence of the different lengths of extraction on growth was tested in the following manner: 100 grams of flour were added to flasks containing 250 c.c. of sterile water and the reaction was adjusted to be neutral to phenol red. The contents of the flasks were digested for four hours at 37° C., and then centrifuged. Thirty cubic centimetres of the watery extract were drawn off and sterilised by the intermittent method at 60° C., three hours' incubation at 37° C. following one hour's sterilisation at 60° C. Each flask was inoculated with one loopful of a thin emulsion of *B. mesentericus* B, and incubated. The rate of growth was estimated by making sub-cultures at 0, 4, 6 and 8 hours' incubation. The sub-cultures were made by inoculating 0·5 c.c. of the experimental fluid into a melted agar tube at 45° C., and pouring as a plate culture. A series of figures obtained in this way is given below:

Table V.

Flour	Primary count	4 hours	6 hours	8 hours
Wholemeal	450	4,020	25,300	—
90 % extraction	400	2,470	12,300	35,000
80 % „	336	2,056	9,040	32,100
80 % „	332	1,900	9,630	28,200
70 % „	408	672	9,430	24,400

These figures suggest that wholemeal flour forms the best medium for growth of *B. mesentericus*, that 90 % and 80 % extraction flours are less favourable for development, and 70 % extraction flours are the least favourable of the flours examined. The differences between the 90, 80 and 70 % extraction flours are not great, but are always of the same order.

Summary.

The skins of grains, all flours and all bread contain bacteria belonging to the group *B. mesentericus*. The cultural characters of six types of *B. mesentericus* isolated from grains and flour are given. Five of these were also obtained from ropy (or sour) bread. None of these five types can be identified as corresponding to the organisms isolated by earlier workers on rope. Rope, or sourness, does not result from the presence of these bacteria unless conditions are such as to allow of great development. The factors determining development of rope in bread are (1) degree of infection, (2) moisture, (3) temperature, (4) reaction, and (5) composition of the flour.

A method of estimating the degree of infection of flours is given.

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