A STUDY OF SOME

WOOL - DISINTEGRATING BACTERIA

By "Animal" (~~Catharine~~ Katrine Horsthouse)

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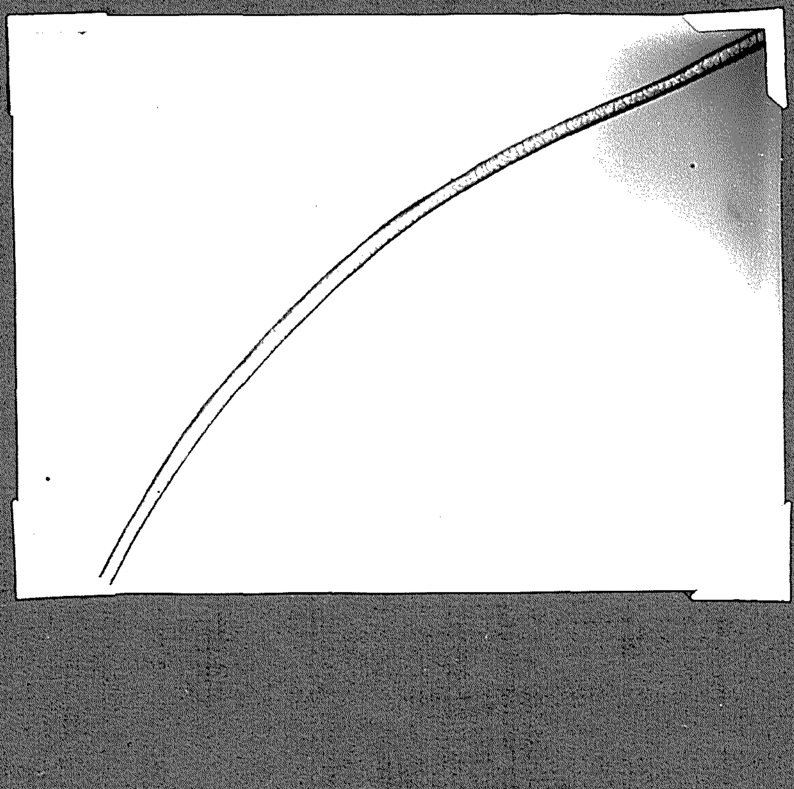
PURPOSE AND SCOPE OF THESIS

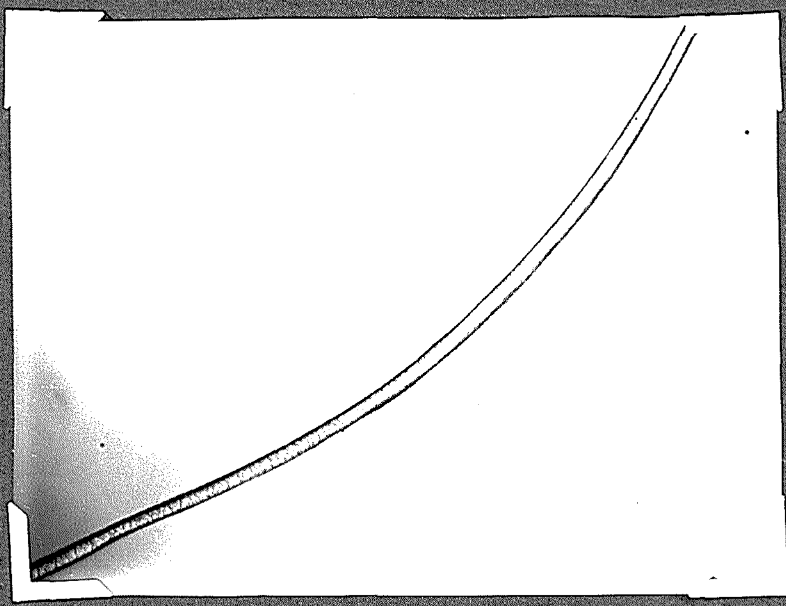
The purpose of this thesis is the isolation and study of the bacteria that ret the wool of living sheep in New Zealand, the study of the distribution of wool-retting bacteria and of some of the conditions in which they bring about decay. It is also desired to show what effe the wool retting bacteria have upon the hoof of sheep - a substance which like wool, consists largely of keratin - and what effect the hoof rotting bacteria have upon wool.

WOOL RETTING.

"Retting" is a word used to describe the process of disintegration of the wool fibre brought about by the activity of micro-organisms. Certain bacteria or their products attack the epithelial scales of the fibre and then the interstitial substance tha binds the cortical cells together. Incipient retting, as it is seen under the low power of the microscope, is characterised by the appearance of longitudinal striations in the fibre. If the retted fibres are pulled in two "brush ends" are seen at the break i.e. the break appears torn and frayed due to the protrusion of the loosened spindle-shaped cortical cells. Sound fibres when pulled in two show a clean even break. The retted fibres lose their strength. In the final stages of retting the fibre loses its form also, the epithelial cells fall away and the cortical cells are loosened.

Burgess and other English workers use the term "tendering" to describe the disintegration of the fibre. In New Zealand wool literature the word "tendering" is applied to the thinning which occur in the wool fibres of some sheep in winter. These "tendered" fibres are much weaker than normal ones. It seems advisable, therefore, to use the word "retting" for the disintegration of wool caused by micro-organisms.





1. Sound wool fibre. The outlines of the epithelial scales can be made out.

Photomicrograph by writer. Magnification × 60

2. Well retted wool fibres. The cortical cells have been liberated. The arrow indicates the epithelial scales.

Photomicrograph by writer. Magnification × 60

Two Samples of Pink Rot Wool.

Photo by Harvey Drake.

Retting is conveniently detected by treating with Pauly reagent. The method described by Burgess and Rimington 1929 is as follows:

To a large test tube add

10 c.c. of 10% sodium salt of sulphanilic acid

5 c.c. of 8% sodium nitrite.

then pour 2 c.c. of concentrated hydrochloric acid slowly down the tube. Mix gently and leave one minute. Immerse the wool in 15 c.c. of 9% sodium carbonate solution and add the mixture. Leave ten minut Wash in water.

Staining takes place only where the epithelial scales of the fibre have sustained damage. Well retted wool samples stain dark brown.

PINK ROT

Retted wool is found in the fleeces of living sheep in the condition known as "pink rot". The rotten wool is sometimes chalky-white but more often coloured pink,yellow,or orange.

Pink rot is found sparingly among New Zealand sheep.

THE ROTTING OF HOOF.

In connection with this thesis some work has been done on a kindred process to that of wool retting - hoof rotting.

Sound sheep's hoof autoclaved at 15 lbs. for 20 minutes and incubated in sterile broth is tough. Its cellular structure cannot be made out from scrapings or unstained free hand sections which appear homogenous and refractive. Broth cultures of some bacteria are able to rot autoclaved hoof. The rotten and gelatinised hoof contains sheets of hoof material in which the cellular structure can be clearly seen in unstained water mounts. Different parts of the hoof rot at different rates - the sole and the pliable horn between the heels are much more readily attacked than the hard external layers

FOOT ROT.

Foot rot was described by French workers (Ludovic and Blaizot) as a contagious chronic disease of sheep characterised by destruction and alteration of the ungual tissue. Experiments by Australian workers, however, cast doubts on the contagious or chronic nature of the disease as it is found in Australia.

Foot rot is extremely prevalent among New Zealand sheep.

REVIEW OF THR WORK ON MICROBIOLOGY OF WOOL AND SOME OTHER KERATINACEOUS SUBSTANCES.

The influence of micro-organisms in the weathering of wool was investigated by Trotman and Sutton 1924. Pieces of woollen fabri were sterilized by boiling in water and then inoculated with cultures of Bacillus subtilis and B.megatherium and incubated at 37·5°C.

Signs of tendering (retting) were distinguished in 48 hours when the outlines of the epithelial cells had become faint. Under prolonged incubation the scales disappeared altogether and in five days the fibre was completely rotten and the spindle-shaped cells of the cortex were liberated. The apparent affinity for acid and basic dyestuffs was increased by retting and after two days' incubation the fibre showed decreased shrinking power.

Burgess 1924, found that retting took place when commerciall scoured wool, sterilized by discontinuous steaming, was inoculated with suspensions of Bacillus mesentericus or B. brevis. Wool improperly sterilized by heating to 100°C in air for 20 minutes, was allowed to dip into sterile distilled water. In 6 weeks a brown coloration was noted where the wool was wet but not saturated.

When examined microscopically the fibres were shown to be badly retted. From this retted wool B.brevis was isolated. B.mesentericus, B.subtilis, B.megatherium, B.brevis, B. fusiformis were all found to ret wool. Retting is found to be greatest when wool is just moist, the degree of decay decreasing as amount of water increases. Local retting appeared in submerged wool. The rotting of commercially scoured wool in this manner with distilled water produced yellowish-brown coloration in the region of moist wool. This was not due to the action of chromogens. The epithelial scales offered greatest resistance to retting since once they were removed or loosened decay proceeded at increased rate.

Wool-retting bacteria were found to grow best in solutions with pH values from 6·3 - 8. Growth was completely checked by pH 5·1.

Trotman and Sutton, 1925, report that spore forming Bacilli that liquefy gelatine, cause extensive damage to wool, destroying epithelial scales and ultimately the whole fibre when the fabric is present in nutrient broth culture of the organisms. The action depen on the reaction of the solution.

With Bacillus subtilis and B.proteus vulgaris, the action is most intense at pH 8. Fabrics so injured have an affinity for dye different from that of undamaged material.

Scoured wool (pH 8 - 8·5) exposed to air but protected from light for two months, became acid in reaction (pH 5 -6) and suffered microscopically visible surface damage. It was found to be infected with B.subtilis, B.mesentericus vulgatus, B.proteus vulgaris, B.mycoides, Micrococcus luteus, M. candicans, a Penicillium and othe organisms.

Burgess, in 1925, showed that at PH 6·5 there was only a slight development of bacteria and fungi. The acid side favoured fun the alkaline, bacteria. Bacteria had no effect at all at 6·5. To hi previous list of retting organisms he added the fungus Cephalothecium roseum and Bact. pyocyaneum.

Burgess 1928, drew attention to the difficulty in sterilizin wool without altering its normal properties. Well washed commercial wool was steamed for twenty minutes in distilled water. The filtered liquid supported a copious fungal growth and the extract obtained afte four successive steamings proved an excellent medium for fungi. It is possible that the presence of a food material more readily available than wool itself may enhance the disintegration of the fibre by an organism which under normal conditions would not cause it.

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Trotman and Sutton found B.proteus vulgaris brought about a very rapid decay of the fibre in presence of nutrient broth. But experiments in Burgess's laboratory failed to credit this organism with rotting wool sterilized by discontinuous steaming and to which no other form of nutrient had been added. Besides the wool retting bacteria, many other species caused the wool to be discoloured but did not appreciably damage the fibre. Wool in moist alkaline condition was readily attacked by bacteria, the spores of which appeared always to be present in wool.

B.mesentericus was able to tender submerged wool when the reaction of the liquid was above pH 6·5 - the degree of tendering was proportional to the alkalinity of the medium up to a point not ascertained.

Burgess 1929, published a paper dealing chiefly with moulds and the enhancement of mildew of wool by soaps and vegetable oils. Often wool supporting a large amount of fungal growth, though discoloured, was not retted. Moulds can thrive in a nearly saturated atmosphere but bacteria require a greater amount of moisture. Bacteri ret wool more rapidly than do moulds. Burgess's work on the enhancement of mildew by soaps, is of interest to the student of the bacterial flora of the fleece, because his methods and findings are suggestive when the relations between yolk,wool and retting bacteria are considered.

Four strips of woollen cloth of exactly equal weight were made to take up soap. Two were incubated with mould spores and two without. Subsequent analyses showed a loss of constituents of soap due to fungal activity. The fatty acid content of the soap was greatl diminished.

Certain fatty acids exhibit a toxic effect on mould growth owing to inhibition of respiration resulting from absorption of these substances on the mycelium. The poisoning capacity increases with molecular weight to caprylic acid and then diminishes. Acids of greater complexity are not absorbed and therefore not toxic. Oleic acid was shown to serve as food for moulds. Nitrogen was detected in aqueous extract of wool cloth made at room temperatures by Biuret reaction and the Ninhydrin test of Abderhalden. The nitrogen requirements of the moulds are fulfilled for the time at least by diffusing substances from wool and these are augmented when proteolyti enzymes of the moulds further hydrolise the wool. If soaps are present they assist hydrolysis of wool on account of their alkalinity.

Burgess and Rimington 1929, describe their method of treating fibres with Pauly reagent - this has already been quoted. They state that when wool fibres are treated with Pauly reagent, staining takes place only where the epithelial scales of the fibre have sustained damage. By application of Totanis reaction, evidence was obtained that tyrosine and histidine in the fibre unite with Pauly reagent to produce a brown-red colour.

The work so far reviewed dealt with rot in wool which had undergone some processing. Trotman and Sutton worked chiefly with fabrics and Burgess with commercially scoured wool.

Waters 1931, drew attention to the occasional occurance of a condition known as "pink rot" in the fleece of living sheep. The condition was found sparingly and in patches about the fleece. It extends upwards two inches from the base of the locks. Affected wool was characterised by its pale pink colour, its matted appearance and rottenness.

Waters continued his work on pink rot and described his results in January 1932. He extended his definition of "pink rot" to include any rotten wool occuring in the living fleece apart from the exposed tips of the locks, whether the affected wool be pink, orange, yellow or chalky-white. He described a bacterium isolated from a sample of pink rot pure cultures of which were capable of rotting sound wool. Samples of sound wool were inoculated with broth cultures of this organism. After four days incubation at 30°C wool showed microscopic signs of retting - it had become opaque, indicating the penetration into the fibres of bacterial products. In ten days the fibres became dull and chalky in appearance with littl or no strength left. The organism was not shown to have any disintegrating affect on the wool cells themselves but rather to dissolve or soften some substance that holds the cells together.

The author describes the organism which does not coincide with all the properties of any of the Bacilli described in Bergey's "Manual of Determinative Bacteriology" but resembles B.vulgatus in most properties and tentatively describes his organism as a variety of B.vulgatus.

The literature on foot rot is scanty. What literature there is deals with the disease from the veterinary point of view with but passing reference to the destruction of the tissues of the hoof. It has been suggested that this is probably a secondary condition arising after the blood supply to the hoof has been cut off as a result of the invasion of the foot by a pathogenic organism. Workers in America and New South Wales describe the anaerobe - Actinomyces necrophorus as the probable causative agent. It has not been shown conclusively that this organism causes foot rot nor that it is directly responsible for the destruction of the ungual tissue.

Jensen 1930, discusses the question of disintegration of keratin by micro organisms. He points out that the scleroproteins generally are characterised by great resistance to action of proteolytic enzymes and by being very little soluble in chemical reagents. Jensen prepared keratin from horn meal, added this to moist field and garden soil and allowed it to decompose in the laboratory.

The keratin was found to undergo a decomposition resulting in a slow but steady accumulation of ammonia and nitrate - 35 to 40% of its nitrogen was transformed into nitrate in 120 days.

Addition of keratin produced little or no increase in number of bacterial colonies on agar platings but markedly increased the number of actinomycete colonies, especially from garden soil. Two strains of actinomycetes were isolated capable of thriving on keratin in pure culture, decomposing it with formation of ammonia.

In 1928 Meunier, Chambard and Comte, published a paper on the pancreatic digestion of wool. Wool samples were soaked in buffer solutions of different pH and were then rinsed in distilled water and incubated with pancreatine. They found that the "protein cement" which holds the spindle-shaped cells together is digested more completely by the pancreatine, the more alkaline the swelling liquor. After swelling at pH 10 at

$18^{o}C$ for 48 hours then incubating at $37^{o}C$ for 48 hours with ·5% pancreatine solution, the wool loses structure altogether without alteration in cellular elements.

Keys 1932, found that pepsin acted on wool, bringing about a disintegration of the fibre. Pepsin is active at about pH 3, which is close to the iso-electric point of wool keratin (pH 3·4 - 3·8) - therefore if the fibre is disintegrated in presence of pepsin, the effect is due to the action of the enzyme unaided by the reaction of the liquid.

Keys immersed a sample of scoured wool in pepsin solution containing a little chloroform. The reaction was adjusted to pH 2·8. The wool and pepsin were incubated at $40^{o}C$

In eight weeks the fibres were appreciably damaged. The scales were obviously loosened. In twelve weeks the fibres were disintegrated when pressed with a cover slip.

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SELECTION, SCOURING AND STERILIZATION OF WOOL FOR TEST PURPOSES.

SELECTION:

For testing the wool retting capacity of pure cultures of bacteria, a good wool (quality 48/50) from a Romney hogget was used.

SCOURING:

The wool was scoured by washing in warm water at about 40°C and then soaking in ether. Scoured wool, even after soaking in ether for a week, was found not to be sterile. Small amounts of this wool were transferred aseptically to sterile petri dishes and sterile broth was poured into the dishes. These were incubated at 30°C. The broth became turbid in 24 hours. This experiment was repeated four times but with the same result. Examination of the contaminated broth after incubation showed the presence of a large number of rod-shaped bacteria, many with conspicuous terminal spores. In spite of the presence of these organisms, the wool remained sound and was not retted when examined in 15 days.

AUTOCLAVED WOOL:

Wool was sterilized by autoclaving at 15 lbs. for 20 minutes.

TIP WOOL AND BUTT WOOL:

The locks of wool were cut in half and butts and tips scoured and sterilized separately. The wool used was shorn wool so that the butt wool did not include the roots.

The wool was put in petri dishes, to some of which water was added and all were autoclaved together at 15 lbs. for 20 minutes.

3 petri dishes containing tips in water
3 " " " tips dry
3 " " " butts in water
3 " " " butts dry

After autoclaving the water was drained off and one of each set examined microscopically.

Of the remainder, four were incubated for 3 days with sterile broth and four with broth cultures of Waters's Pink Rot organism - called "Prosp" in this thesis.

The results of microscopic examination were as follows:-

Material	After Autoclaving	After 3 days incubation with sterile broth.	After 3 days incubation with Prosp.
Tips Wet	Much twisted and yellowed.	Much twisted and yellowed.	Well retted.
Tips Dry	Fibres clear or yellowed.	Fibres clear or yellowed.	Well retted.
Butts Wet	Slightly twisted and slightly yellowed.	Slightly twisted and slightly yellowed.	Retted.
Butts Dry	Fibres mostly sound and clear.	Fibres mostly sound and clear.	Retted.

From the above it will be seen that butt wool is less affected by autoclaving than tip wool and that both are altered more when autoclaved in the presence of water than without.

Waters had noted previously that tip wool is more easily retted than butt wool. This is probably due to weathering. The tip of the lock is, of course, older than the part near the skin and has occupied a more exposed position in the fleece. The yolk is washed from the tip of the lock by rain and leaves the fibre exposed to light and atmosphere.

Trotman and Sutton 1924, quote Kerterz who concludes that all the effects of weathering could be produced by actinic light alone, but they state that air containing ozone attacks the epithelial scales producing soluble sulphur and increased percentage of soluble nitrogen.

Sommer 1928, holds that the destruction of fibres on weathering is due to ultra violet rays. Deterioration is assisted by moisture. That the tip is altered by weathering is shown by the fact that it stains with carbol fuchsin or Pauly reagent, while the butt wool remains colorless.

EFFECT OF STERILIZATION ON THE FIBRE.

In a search for a good method of obtaining sound steril wool for test purposes, the effect on the fibre of various methods of sterilization was tried. It was desired to cause as little alteration as possible to the fibre in order that laboratory experiments on the retting of sterile wool by cultures of various organisms might give an insight into the naturally occurring pink rot of wool.

Broth cultures of Prosp were added to butt wool partially sterilized by scouring in warm water and soaking in ether, and to butt wool similarly scoured in warm water and ether and autoclaved dry. Both cultures were incubated at 30°C

In 24 hours neither showed signs of retting.

In 2 days the fibres of both were discoloured and showed the longitudinal striations indicative of incipient retting.

In 4 days retting was well advanced in both. The autoclaved wool was not retted noticeably faster or slower than the unautoclaved but as the unautoclaved wool was contaminated by the presence of more than one kind of organism, the actual effect of the autoclaving was obscured.

It seemed probable that the fibre is considerably altered in its chemical and physcial nature by the heat and presence of autoclaving.

Following Burgess's example, therefore, the method of discontinuous steaming was tried.

The wool was put in test tubes, plugged with cotton wool, and sterilized by heating for 20 minutes in a steamer on each of three consecutive days.

THE INFLUENCE OF WATER AND YOLK ON STEAMED AND ON AUTOCLAVED BUTT WOOL.

Some experiments were set up on the sterilization of unscoured wool. The wool was sterilized in test tubes·

Some was sterilized in presence of water. Water was added to the remainder after sterilization . The tubes were incubated at 30°C.

Some were examined after 2 weeks, the remainder after 4 weeks. The state of the fibres was shown by treating with Pauly reagent. The staining properties did not alter with the time. Six tubes of each class were examined and the results were uniform.

Material	Pauly Reaction.
Autoclaved wet unscoured	dark orange brown
Autoclaved wet scoured	no stain.
Autoclaved dry unscoured.	orange.
Autoclaved dry scoured	no stain.
Steamed wet unscoured	patches of orange.
Steamed wet scoured	no stain.
Steamed dry unscoured	no stain.
Steamed dry scoured	no stain.
Unsterilized unscoured	no stain.
Unsterilized scoured	no stain.

These results are not surprising. The combined action of heat, pressure, water and yolk (yolk contains a large quantity of Potassium salts - the free alkalie is probably liberated) have profoundly altered the fibre in the "autoclaved, wet, unscoured" series. Heat, pressure, yolk and heat, water, yolk have also modified the staining properties of the fibre.

CONCLUSIONS:

In the light of the foregoing experiments, a standard method in the selection and sterilization of wool for test purposes was adopted.

Butt wool - the first 2 inches from the cut end of the lock - was used. This was scoured in warm water and ether and sterilized by heating in test tube in the steamer for 20 minutes on each of three consecutive days. Wool treated in this manner is hereafter referred to as "test wool".

However, the early retting tests were made before these experiments were completed. Some were carried out on scoured wool improperly sterilized by soaking for several days in ether and transferred aseptically to sterile petri dishes. Apparently the wool retting organisms present in the fleece were unable to withstand the soaking in ether, as the control experiments in which sterile broth was added to scoured wool, always remained sound for the duration of the retting test (15 days).

Other tests were carried out on scoured wool autoclaved in petri dishes.

Tip wool, or wool autoclaved wet, or unscoured wool, was never used for test purposes.

ISOLATION OF WOOL RETTING AND HOOF ROTTING BACTERIA.

I. FROM PINK ROT WOOL.

Method of isolation:

Small pieces of pink rot wool were shaken up in steril water. A loopful of the suspension was plated on standard nutrient agar, using a fine, sterile, bent glass rod to spread the liquid over three plates. These direct platings showed a varied flora.

Yellow chromogens were always present and pink ones were often abundant. Five isolations were made from the first two samples examined. Of these one formed small pink colonies, two formed yellow colonies, and two cream colonies on agar. Broth cultures of these organisms and one of Prosp were added to scoured wool and incubated at 30°C. The wool to which a culture of Prosp had been added was completely retted in 4 days. The wool in the other cultures and in the controls to which sterile broth had been added, were sound after 15 days. No colour developed in the broth or on the wool, though the organisms grew in broth causing turbidity.

Having failed to isolate an organism from direct platings , an "enrichment" scheme was tried. Small pieces of the pink rot wool were shaken up in broth, the wool allowed to settle and the liquid added to autoclaved wool. The wool was well retted in a week. Loopfuls of the broth in which the woo was retted, were plated out.

The colonies present on the plates after enrichment were strikingly different from the direct platings. Chromogens were absent and large, spreading colonies predominated. One isolation from each of two different pink rot samples was made and both, after purifying by plating out, were found capable of retting wool in broth.

II. FROM DISEASED HOOF MATERIAL.

It is customary to pare the feet of sheep suffering from foot rot in order to expose the necrosed tissues to the air. Some parings were collected in sterile petri dishes in the sheds where paring was in process. The pieces of hoof were all more or less rotted and noticeable for their offensive smell.

Pieces of sound hoof for test purposes were sterilized by autoclaving at 15 lbs. for 20 minutes. Representative pieces were cut about 1 inch by 1/3 inch, including a piece of the sole and a piece of the hard horny layer of the toe.

Method of Isolation from Foot Rot Parings:

The same method was used as in the isolation of wool retters. Gross innoculations into broth were made from selected parings. These were added to sound,unautoclaved hoof and incubated at 30°C. Rotting was evident in 6 days. Eight isolations were made from platings of the "enriched" cultures. Of these, one was able to rot autoclaved hoof in pure culture in broth. This organism was shown to ret test wool completely after 10 days incubation in broth culture at 30°C. It slowly produced small,circular,yellow colonies on agar. Stained smears from these colonies showed the presence of small rods. The organism is referred to as F_3.

Two conspicuous white colonies appeared on a sparsely populated plate made from a white fluid carefully collected from a lesion in a badly diseased hoof. Microscopic examination showed that the organisms forming the colonies were large, spore bearing rods. An isolation was made from one colony and the purified culture was found to have slight wool retting and hoof rotting powers in the presence of broth. This bacillus will be referred to as F_1.

III. FROM CRUMBLING MATERIAL FROM HEALTHY HOOFS.

A crumbling of the sole of the hoof very frequently takes place under the overgrown toe. This is not a pathogenic condition. White material can be collected by scraping. Microscopic examination shows this material to consist of masses of isolated hoof cells. After enrichment, a spore bearing rod which formed characteristic colonies on agar was isolated. The purified culture was found to rot autoclaved hoof and wool in the presence of broth. Reference number F_2.

The exact conditions under which the organisms isolated ret wool, are described in the next section, while the reactions of most of them are described on page 37.

RETTING TESTS.

The results of early tests made at different times and for different periods of incubation are given in Table I. The trials were carried out on wool autoclaved in petri dishes. Two inch lengths of scoured wool were placed in petri dishes and covered with 3 inch by 1 inch glass microscope slides to keep the wool flat. Cultures of the organisms to be studied were purified by plating out. Broth cultures were added to the autoclaved wool and care was taken to tap the glass slide with sterile forceps until the liquid had penetrated underneath it.

The plates were incubated at 30° C. They were examined under the low power of the microscope (2/3 inch). The lids were taken off the plates which were placed on the stage and the fibres examined "in situ" under the glass slide.

I regarded the fibres as completely retted when every fibre or almost every fibre was discoloured and marked with longitudinal striations. Such wool had invariably lost its strength and showed brush ends when torn. In every case the controls to which sterile broth had been added,remained sound.

When many retting tests are being made and the wool cultures have to be kept for a long period, petri dishes are cumbersome and liable to contamination. Therefore in later work I used test tubes plugged with cotton wool in which 2 inch lengths of scoured wool were sterilized by discontinuous steaming.

Tubes containing 5 c.c. of sterile broth or distilled water were lightly innoculated with a platinum loop from a turbid broth culture of the organism to be investigated. The water or broth thus innoculated was poured into a tube containing wool. The lips and plugs of both tubes were carefully sterilized by flaming. The flame was extinguished with a cloth wet with corrosive sublimate, the plugs pulled out a little further and flamed and extinguished again.

The broth and wool cultures were made in duplicate with two controls and incubated at 30°C. One tube of each was opened after 7 days and the fibres treated with Pauly reagent. The second tube was similarly examined in 10 days.

Four wool and water cultures of each organism and eight controls (sterile wool to which sterile water was added) were made. These were capped with rubber caps to prevent loss of water by evaporation and incubated at 30°C.

In both broth and water experiments, the wool was partly but not wholly, submerged giving the bacteria a chance to attack the submerged wool or the wool at or near the surface of the liquid. One of each of the wool and water cultures was opened and the fibres treated with Pauly reagent after 3 weeks, one of each after 5 weeks, and the remaining two after 6 weeks 4 days.

The results of retting trials on test wool with both water and broth cultures and controls are shown in Table II. The following abbreviations are used :

R = Retted. All the fibres throughout their length or all the fibres for part of their length are stained orange-brown with Pauly reagent. The fibres have lost their strength and when torn, show brush ends.

sR = Slightly Retted. A few fibres are darkly stained with Pauly reagent and show brush ends when torn.

S = Sound. No fibres are darkly stained with Pauly reagent. There is no weakness in the fibres but clean breaks occur when the fibres are torn. Some fibres have taken patches of pale yellow stain.

After treating with Pauly reagent samples in class R can readily be distinguished from the others without microscopic examination; but samples of the sR and S classes may both appear pale yellow or may both appear white. The distinction is based on the presence in the sR class of an occasional darkly stained fibre or brush end, visible under the microscope.

PINK ROT SAMPLES.

Sample No.1

Collected from under elastic bands on sheep covered for experimental purposes, in the Manawatu District.

Sample No.2

Collected in November 1931, from hoggets at Longburn, New Zealand.

TABLE I.

RESULTS OF PRELIMINARY RETTING TESTS WITH WOOL AUTOCLAVED IN PETRI DISHES AND IMMERSED IN INOCULATED BROTH.

Organism	Source from which organism was isolated.	Retting capacity.
Prosp	Isolated by Waters from pink rot sample No.1.	Repeated trials show retting complete in 3 days.
W_1	Isolated by Waters from pink rot sample No.1 but its retting powers were unknown.	2 plates examined 5 and 26 days respectively - both completely retted.
W_2	Isolated by Waters from pink rot sample No.1 but its retting powers were unknown.	2 plates examined 8 and 26 days respectively. No retting in either.
W_3	Isolated by writer from pink rot Sample No.1.	3 plates examined 5,10, and 24 days respectively Completely retted in eac
W_4	Isolated by writer from pink rot sample No.2.	3 plates examined 5,10, and 24 days respectively Completely retted in each
F_1	Isolated by writer from colony appearing on foot rot platings.	2 plates examined 7 days and 4 weeks. An occasional fibre retted 7 days. Well retted (with occasional sound fibre) in 4 wk
F_2	Isolated by writer from white, crumbling material from healthy foot of sheep.	2 plates examined 5 day and 4 weeks respectively Both completely retted.
Bacillus vulgatus.	American type culture	1 plate examined 12 day No retting.
B.mycoides.	American type culture	2 plates. One examined1 other 14 days. Very slight retting in both.
B.megatherium.	Laboratory culture	2 plates. One examined 1 days, other 24 days. Very slight retting in both.

TABLE II.

RETTING TESTS WITH TEST WOOL IN INOCULATED BROTH AND IN INOCULATED DISTILLED WATER.

Organism used as Inoculum	Broth. 7 days Tube 1.	10 days Tube 2.	Distilled water. 3 weeks Tube 1.	5 weeks Tube 2.	6½ weeks. Tube 3.	Tube 4.
Prosp	R	R	sR	sR	R	R
W_1	R	R	sR	sR	R	sR
W_2	S	S	S	S	S	S
W_3	R	R	sR	sR	R	R
W_4	sR	sR	sR	sR	R	R
F_1	sR	S	S	Cultures lost		
F_2	sR	sR	S	R	sR(5 wks)	sR
Controls	S	S	S	S	S	S
Controls	-	-	S	S	S	S
B. vulgatus	-	S	-	sR	sR	-
B. mycoides	-	sR	-	sR	sR	-
B. megatherium	-	sR	-	sR	R	sR.

It will be seen from Table II that with one exception, advanced retting did not occur in wool and water tubes in 5 weeks but it did occur in the presence of Prosp, W_1, W_3, W_4, and B. megatherium in 6½ weeks. The results obtained with F_2 were odd. Of the two tubes opened in 5 weeks, one was rotten, while the other was only slightly retted. A loopful of water from each tube was plated on agar and incubated. Platings from both tubes showed a plentiful growth of typical F_2 colonies.

Coloration of Retted Wool.

No colour was developed on any of these cultures. Burgess 1924, noted a brown coloration appearing on commercially scoured wool that had been heated to 100°C in air and allowed to dip into distilled water for 6 weeks. Bacillus brevis was isolated from this wool, which was badly retted. Burgess states that the coloration was not due to chromogens.

My experiments show that natural wool scoured in warm water and ether and sterilized by steaming, shows no discoloration whatever in 6½ weeks when retted by pure cultures of certain spore bearing Bacilli nor by a mixture of soil organisms (page 32).

It seems probable that the brown coloration noted by Burgess as accompanying bacterial disintegration, is a result of the processing to which his fibres had been submitted previously, unless B.brevis has a markedly different action on wool from the Bacilli studied by me.

Further attempts were made to reproduce the pink colour which so frequently accompanies the retting of wool in the living fleece.

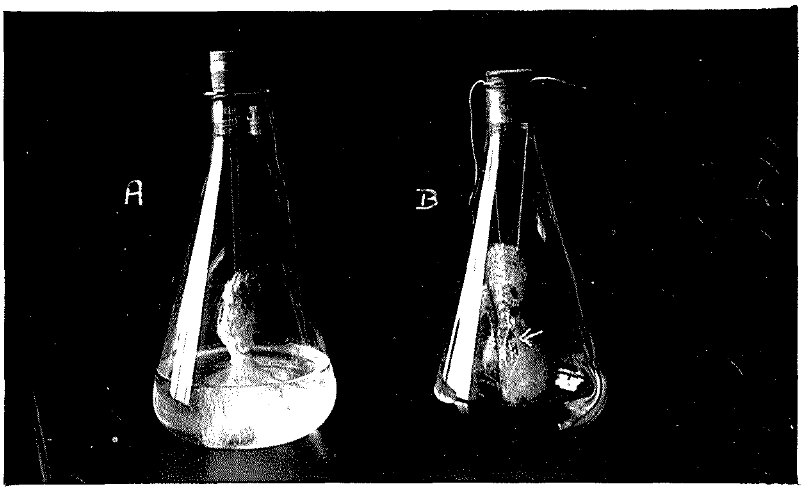
Two chromogens were isolated from a pink rot sample, freshly collected by the writer. The pink rot occurred at openings in the fleece made by elastic bands tied round the sides and belly of the animal to keep a small canvas cover on the back.

One of these chromogens was dark red, the other pink, on agar slopes. Turbid broth suspensions of these bacteria were added to test wool; also a mixture of a water suspension of a chromogen and a water suspension of Prosp thus :

Dark red chromogen and sterile, distilled water and wool.
" " " " Prosp and sterile distilled water and wool.

Pink chromogen and sterile distilled water.
" " " Prosp and sterile distilled water.

Examined after 6½ weeks, a pink or red sediment had accumulated at the bottom of each tube, but in no case was the wool coloured. The wool in the tubes to which suspensions of Prosp had been added, were slightly retted, but the wool remained white. The chromogens might, of course, have coloured the wool in advanced stages of retting.


A
B

PHOTOGRAPHS OF FLASK EXPERIMENTS.

A. The flask contains two locks of wool partly immersed in water.

B. The flask contains two locks of wool to each of which a piece of pink rot is tied (see arrow).

Photo by Gabriel.

ACCOUNT OF SOME
GENERAL EXPERIMENTS ON ROTTING OF WOOL .

Experiments were set up to investigate some of the factors which might affect the retting of wool in nature.

Wool was obtained in August, shorn from sheep at end of July. It was Romney hogget wool and obtained from the shoulder. A quantity was collected in the wool shed in a sterile container. In order to avoid possible contamination from the air in the laboratory in which pink rot was being investigated, the wool was prepared for the experiment in a lecture room. Benches and hands were swabbed with corrosive sublimate.

The wool was separated into small locks. The average length of these was about 6 inches, though they varied from 5 to 8 inches. The locks stained with Pauly reagent for approximately 2 inches from the tip. The tip of the wool belonged to the birth coat of the animal.

The locks were bent double and tied loosely in this position. Small pieces of pink rot about ½ inch in length and collected from living sheep immediately prior to the experiment, were used to inoculate some of the locks. The pieces of pink rot were slipped under the cotton that tied the lock and thus came in contact with the wool 1½ - 2 inches from the butt and about the same distance from the tip. Pieces of thread 18 inches long were cut and the locks threaded on these in pairs. The pairs of locks received various treatments and were suspended in autoclaved 500 c.c. flasks over water, or partially immersed in water and incubated at 30 C. All locks whose treatment is designated as "humid" or "sprayed", were suspended over water. Locks immersed had about 1½ inches of the tip and the same amount of the butt dipping into liquid in the bottom of the flasks. The flasks were corked with rubber bungs. They contained each a pair of unsterilized locks of wool hanging over water, or partly immersed in sterile water in a sterile atmosphere.

The treatment received by each pair of locks is given below:

FLASK EXPERIMENTS.

Without added Inoculum:

H P C	Humid Plain Control	Locks without inoculum.
S P C	Sprayed " "	Locks sprayed with water.
1 P C	Immersed " "	Locks partly immersed in water.

With added Inoculum of Pink Rot;

H P 1	Humid Plain Inoculated	Treatment as for corresponding "control" series with addition of pieces of pink rot wool.
S P 1	Sprayed " "	
1 P 1	Immersed " "	

Dipped and without added Inoculum:

H D C	Humid Dipped Control	Locks dipped in Little's Fluid dip 1/100 strength for 1 minute, dried in dessicator for 1 day and treated as for "plain control" series.
S D C	Sprayed " "	
1 D C	Immersed " "	

Dipped and with added Inoculum of Pink Rot:

H D 1	Humid Dipped Inoculated	Locks treated as for "Dipped Control" series with addition of pieces of pink rot as inoculum.
S D 1	Sprayed " "	
1 D 1	Immersed " "	

Treated with Soil:

The soil was taken from under the turf in a grazing paddock.

H S	Humid Soil	Locks well rubbed in slightly damp soil.

S S	Sprayed Soil	Locks sprayed with soil and water suspension.
I S	Immersed Soil	Lumps of earth were added to the water in the bottom of the flask and stirred. Locks partly immersed in this liquid.

Treated with Sheep's Dung:

The dung was freshly collected from a grazing paddock and kept for 2 days.

H D	Humid Dung	Locks rubbed in dung.
S D	Sprayed "	Locks sprayed with dung and water suspension.
I D	Immersed "	Locks partly immersed in dung and water.

Treated with Prosp:

H P	Humid Prosp	Locks sprayed with a turbid suspension of Prosp, dried in a dessicator for 1 day and then put in humid atmosphere in flask.
S P	Sprayed Prosp	Locks sprayed with turbid suspension of Prosp.
I P	Immersed Prosp	Locks partly immersed in turbid suspension of Prosp.

One lock from each flask was removed at end of 2 weeks and examined with Pauly reagent. After 4 weeks the remaining lock was split lengthways, half was treated with Pauly reagent, and half allowed to dry.

Beads of moisture appeared after incubation on the locks that had not been wetted before incubation. This was probably due to the hygroscopic properties of the yolk. Changes of temperature caused by opening the incubator would produce condensation also.

All the locks stained with Pauly reagent for about 2 - 3 inches from the tip, due to previous weathering. Staining of butt wool or rotting of any part of the lock, except the very tip, were noted as they were brought about by the conditions of the experiment.

The word "rotting" is used in describing the wool in these experiments as it is a more general term than "retting". The fibres were not examined microscopically - the staining properties of the locks were noted and their strength judged and taken as a measure of the alteration they had undergone.

The butt is the first $1\frac{1}{2}$ - $2\frac{1}{2}$ inches.

The tip is the last $1\frac{1}{2}$ - $2\frac{1}{2}$ inches.

The loop is the region about 1 inch on either side of the bend. In the immersed samples, the loop alone is above water.

RESULTS OF FLASK EXPERIMENTS.

	After 2 weeks incubation.	After 4 weeks incubation.
Without added Inoculum:		
H P C	Butt 2inches unstained. Rest rotten.	Stained and rotten throughout.
S P C	Tip rotten. Rest sound.	Butt lightly staine for 1 inch. All moderately rotten.
I P C	Sound throughout.	Sound $1\frac{1}{2}$ inch butt rotten at bend, al immersed wool fair sound.
With added Inoculum of PINK ROT:		
H P I	Butt 2 inches unstained. Rest rotten.	Stained and rotten throughout.
S P I	$\frac{1}{2}$ inch at butt stained, $1\frac{1}{2}$ inch unstained. Rest rotten and stained.	Stained and rotten throughout.
I P I	Butt 2 inches unstained rotten loop(above water) sound tip(below water)	Butt $1\frac{1}{2}$ inches unstained,rotten at loop, tip sound.

RESULTS OF FLASK EXPERIMENTS (contd.)

	After 2 weeks Incubation.	After 4 weeks incubation.
DIPPED and without added Inoculum:		
H D C	Sound throughout.	Sound throughout.
S D C	Butt 2 inches sound, rest rotten.	Sound throughout.
I D C	Butt 2 inches unstained, rest slightly rotten.	2 inch butt sound, rest slightly rotten
DIPPED and with added Inoculum of PINK ROT:		
H D I	Sound throughout.	Nowhere much rotted.
S D I	Sound throughout.	Nowhere much rotted.
I D I	Slightly rotten at loop, otherwise sound	Rotten at loop. Tip and butt sound.
Treated with SOIL:		
H S	Butt sound. Rest rotten.	Stained and rotten throughout.
S S	Not appreciably rotted.	Lightly stained and somewhat rotten throughout.
I S	Tip and butt lightly stained. Thoroughly rotten in submerged parts, but not at loop.	All submerged parts thoroughly rotten. Loop somewhat rotten
Treated with SHEEP'S DUNG:		
H D	Rotten throughout.	Rotten throughout.
S D	Moderately rotten	Rotten throughout.
I D	Almost completely sound.	Almost completely sou but slight rotting at loop.
Treated with PROSP:		
H P	½ inch at butt stained, 1 inch sound, loop and tip rotten.	Butt lightly stained loop & tip rotten.
S P	Small patches at butt rotten, 1 inch sound, tip & loop rotten.	Rotten throughout.
I P	Tip and butt practically sound. Loop rotten.	Some sound butt wool but rest rotten.

Incubated dry with PINK ROT)
Incubated dry without Inoculum)

Butt unstained.
Tip and loop stained but not rotten.

DISCUSSION OF RESULTS.

The experiments showed clearly that given constant conditions of temperature and moisture, the butt wool rots much more slowly than the rest of the lock. Locks kept dry and not attacked by organisms, were stained deeply by Pauly reagent at the tip, the colour perceptibly lessening with distance from the tip and ceasing 2 inches to 3 inches from the base of the lock. Presumably the weathering of the tip made it more easily attacke

After 2 weeks the butt was sound and unstained in most of the samples. A small rotten patch at the base of the lock was noted in three of the samples incubated for a fortnight. Examination of the samples of 4 weeks incubation, showed that th last part of the lock to remain sound was the region from ½ inch to 1½ inches from the base.

The locks that were half immersed in liquid, with the exception of those in the soil solution, rotted more slowly than the others. Rotting began at the loop which was above water, while the immersed tip and butt remained sound or only slightly rotten.

Locks immersed in dung and water remained sound but both locks immersed in soil solution were conspicuously rotten. After the experiment the pH of the dung solution was 6·8, that of the soil solution 6·7.

The locks that had been dipped were less affected than the others. When setting up the experiment, I noted that the dipped locks were wetted with difficulty. It may have been this difference in the water absorbing power of the wool and not the bactericidal action of the dip that protected the wool.

The experiments did not show clearly that there was a marked increase in the rate of decay of the locks inoculated with pink rot or wetted with suspensions of Prosp, compared with the controls.

No pink or yellow colour developed on any of the locks. The pieces of wool that were incubated for 4 weeks and allowed to dry without staining, showed a white chalky matted appearance where they were rotten, but no development of colour.

Unscoured wool, sterilized by steaming and incubated in water at 30° for 4 weeks, is not rotted. It does not seem likely that wool which is not chemically disintegrated by steaming and incubation in the presence of yolk (see page 15) will be chemically disintegrated by yolk without the application of heat. I presume that the decay of the fibres in these experiments was due to the activity of organisms and conclude that wool-retting organisms were present in the wool collected in the wool shed.

DISTRIBUTION OF WOOL-RETTING BACTERIA.

The Soil.

Some soil was collected in a sterile flask from under the turf in a grazing paddock. A few cubic centimetres of a water suspension of this soil were put in a centrifuge tube. The suspended particles were thrown down by centrifuging briefly and the turbid supernatant liquid was drawn off and centrifuged for 10 minutes. A sediment was thrown down. The clear liquid was drawn off and the sediment washed by stirring up with a few c.c. of sterile water and again centrifuged for 10 minutes. The process was repeated once more. Stained smears of the final sediment examined under the microscope, showed masses of bacteria. These had been washed free from soil particles and dissolved substances. A turbid water suspension of the sediment was added to four tubes containing test wool. The tubes were capped to prevent loss by evaporation and incubated at 30°C.

One tube was opened in 4 weeks and the wool treated with Pauly reagent. A few fibres were stained by the reagent and an occasional brush end was seen. The wool in the three remaining tubes examined in 6½ weeks, was completely rotten and very darkly stained by Pauly reagent. Controls in which sterile water was added aseptically to test wool, were sound when examined in 4 weeks.

Wool-retting organisms have been shown to be present in soil collected from a grazing paddock and there is every indication that wool-retting micro-organisms were present in an unscoured sound fleece stored in a wool shed (page 31).

The bacteria - F_1, F_2, and F_3 isolated from disintegrating hoof, proved able to ret wool as well as hoof.

THE ROTTING OF WOOL AND OF HOOF.

The following is a list of the organisms studied and of their ability to rot autoclaved wool and hoof in broth culture after incubation at 30°C.

Organism.	Scource from which organism was isolated.	Autoclaved Wool & Broth Cultures Incubated 15 days.	Autocalved Hoo[f] & Broth Culture Incubated 15 da[ys]
Prosp	Pink rot	Completely retted	Rotted
W_1	" "	" "	"
W_2	" "	Not retted	"
W_3	" "	Completely retted	"
W_4	" "	" "	"
F_1	Foot rot parings	Partially "	Slightly rotten in 24 days.
F_2	Crumbling hoof	Completely "	Rotted.
F_3	Foot rot parings	" "	"
B.vulgatus	Type culture	No retting in 12 days.	"
B.mycoides	" "	No retting in 12 days.	"
B.megatherium	Laboratory culture.	Slight retting in 12 days.	"

THE EFFECT OF YOLK ON WOOL AND WOOL-RETTING BACTERIA.

Burgess 1929, has shown that the presence of soaps on commercially scoured wool encourages the growth of moulds which use the soap as food and later attack the fibre.

Considering the soapy nature of yolk, an attempt was made to find out what relation it bore to the retting of the fibre

The Effect on the Fibre of varying concentrations of Raw Yolk in Water:

Twenty grams of the good Romney wool used in previous experiments was packed tightly into a 6" x 2" test tube, in the bottom of which were a number of small pieces of glass tubing to prevent the wool from being forced into the bottom of the tube. 10 c.c. of water were added to the tube which was corked and centrifuged in a Gerber centrifuge to 1200 rotations a minute. The tube was reversed with the base towards the centre and centrifuged again. By repeating this process and passing the water five times through the mass of wool, a very thick and concentrated yolk extract was obtained. This was drawn off and a series of dilutions made in sterile Durham tubes. Two inch lengths of ether scoured butt wool from the same source as that used for yolk extraction, were placed in each tube. The tubes were corked with rubber bungs and incubated at 30°C - layer of wax had appeared on the surface of the first three dilutions when examined in 2 days.

Some fibres were drawn off after 2 days. These were sound and unstained by Pauly reagent. The remainder were withdrawn in 3 weeks. They were not discoloured. They were washed in water and treated with Pauly reagent.

Effect on Wool of 3 weeks' Incubation in Yolk Dilutions.

No.	Dilution.	Staining Reactions	Strength.
1	Full strength	Orange-brown	Sound
2	75%	Dark-brown	Slightly retted
3	50%	Orange-brown	Sound
4	25%	Orange-brown	Sound
5	1%	Unstained	Sound
6	Distilled water	Unstained	Sound.

The staining of the fibres noted in this experiment was not apparently due to retting brought about by organisms present in the unsterilized yolk. Butt wool incubated with retting organisms in distilled water only was not stained deeply by Pauly reagent until the fibres had lost all their strength and showed brush ends whenever they were torn. With the exception of the wool in the 75% dilution where there was some evidence of retting the stained wool in the experiment just described had not lost its strength and showed clear breaks when torn. Perhaps the yolk in solution or the hydroxyl ions had a chemical effect on the fibre which changed its staining reactions.

Autoclaved Suint.

Concentrated solutions of autoclaved suint were found to inhibit the growth of Prosp in broth cultures. Water-soluble yolk extracted, evaporated to dryness, and weighed in the chemical laboratories of this College, was used.

Twenty c.c. of distilled water were added to 1·39 grams of dried suint and dissolved by autoclaving. The solution was diluted with broth. A set of test tubes was arranged containing a series of broth and suint dilutions. There was 5 c.c. of liquid in each tube. The tubes were plugged, autoclaved, inoculated from a 5 hour broth culture of Prosp, and incubated at 30°C. The dilutions were examined in 1 day.

The results are given below:

Dilution	Composition of Dilutions	Observed in 1 day
Full strength	No broth	No signs of growth
½	1 part soln.: 1 part broth	" " " "
¼	1 part soln.: 3 parts broth	" " " "
1/8	1 part soln.; 7 parts broth	" " " "
1/16	1 part soln:15 parts broth	" " " "
1/32		turbidity
1/64		"
1/128		"
Broth		"

The experiment was repeated in a rather different manner.

1·9 gms, suint were dissolved in 20 c.c. of distilled water, the solution was filtered and diluted with water. A series of tubes were arranged containing each 5 c.c. of different suint and water dilutions. To each of these 5 c.c. of broth was added. The tubes were plugged, autoclaved, inoculated from 5 hours broth cultures of Prosp and incubated at 30°C. Each tube contained the same amount of broth and 5 c.c. of suint solution of varying strength. Observations made after 1 day and after 5 days are given below :

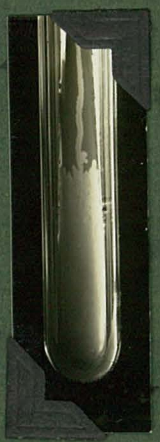
Dilution	Observed in 1 day	Observed in 5 days
Full strength & broth	No signs of growth	No signs of growth .
½ " " "	" " " "	" " " "
¼ " " "	" " " "	Light pellicles
1/8 " " "	" " " "	Pellicle
1/16 " " "	" " " "	"
1/32 " " "	Turbid	"
1/64 " " "	"	"
1/128 " " "	"	"
Water " "	"	"

Conclusion:

1·9 grams of dried suint, dissolved in 20 c.c. water, filtered, and diluted to ¼ strength, was found to inhibit the growth of Prosp when added to an equal volume of broth and incubated with the organism for 5 days at 30°C. Much lower concentrations of autoclaved suint markedly retarded the growth of this organism.

The conditions of this experiment, however, were too artificial and the treatment of the suint too drastic for this fac[t] to have any real bearing on the problems of bacterial food supply in natural unscoured wool.

It was not thought possible to sterilize water extracts of raw yolk by filtration as the waxes of the yolk were present as an emulsion.



PROSF

W[illegible]

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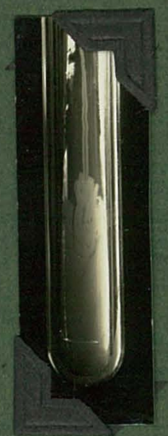
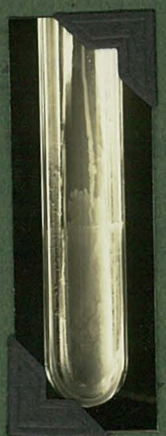


W3



W4

F1

F2

Retting organisms on agar slopes after two days' incubation at 30°C.

Photo by Gabriel.

REACTIONS OF RETTING BACTERIA.

Seven cultures were chosen for further study. Prosp, isolated by Waters and described by him, was put through its reactions for the purposes of check and comparison.

1.	Prosp	Isolated and studied by Waters. A good wool and hoof retter (autoclaved hoof and broth).
2.	W_1	A good wool and hoof retter(autoclaved hoof and broth)
3.	W_3	" " " " " " " " " "
4.	W_4	" " " " " " " " " "
5.	F_2	" " " " " " " " " "
6.	W_2	Rots autoclaved hoof in broth but not wool.
7.	F_1	A poor retter of autoclaved wool and autoclaved hoof in broth.

These cultures were purified by plating out before beginning the tests. The Manual of Methods and the Descriptive Charts prepared by the Committee on Bacteriological Technic of the Society of American Bacteriologists were used and their method followed closely.

Prosp and W_3 are very much alike in all their reactions. They were isolated from the same source and are indistinguishable on agar slopes or plates.

W_1 and W_4 isolated from different pink rot samples are also much alike. I find it possible to distinguish them by examining their agar slopes. W_1 and W_4 also resemble Prosp and W_3 closely.

Prosp	W_3
Isolated by Waters from pink rot.	Isolated by writer from pink rot.
Short rods arranged singly and in pairs. Ends rounded.	Short rods arranged singly and in pairs.
Size of majority $1{\cdot}9\mu \times {\cdot}8\ \mu$ (from agar slopes incubated at 30^o for 1 day)	Size of majority $1{\cdot}9\mu \times {\cdot}7\mu$ (from agar slopes incubated at 30^o for 1 day)

(contd.)

Prosp	W3.
Sporangia	
Present of same size and form as vegetative cells. Found sparingly in agar cultures more than 1 day old. Do not stain easily.	Of same size and form as vegetative cells. Found sparingly on agar cultures more than 1 day old. Do not stain easily.
Spores	
Central to excentric. The shed spores seen as refractive, unstained elipsoids or cylinders in smears from 4 day agar slopes stained with carbol fuchsin (without fixing or washing)	Central to excentric. Spores seen as refractive unstained elipsoids or cylinders in smears from 4 day agar slopes stained with carbol fuchsin.
Motility	
Maximum motility in broth 5 -18 hours. Film from 17 hours agar slope stained with Gray's Flagellum stain. Flagella peritrichous - usually 16.	Maximum motility in broth 5 - 24 hours. Film from 17 hours agar slope stained with Gray's flagellum stain. Flagella peritrichous - about 16 in number.
Gram positive becoming negative on 3 rd day.	Positive becoming negative on 3rd day.
Agar Stroke. At 30^{o}C for 1 day. Growth moderate, spreading, slightly raised, smooth, opaque, butyrous.	At 30^{o}C for 1 day. Growth moderate, spreading, flat glistening, smooth, opaque, butyrous.
Gelatine . At 20^{o}C. Liquefaction crateriform Begins in 2 days, complete in about 5 weeks.	At 20^{o}C. Liquefaction crateriform. Begins in 2 days - complete in about 4 weeks.
Gelatine Colonies. 20^{o}C for 3 days. Growth slow, irregular or circular, edge undulate, liquefaction spreading.	20^{o}C for 3 days. Growth slow, irregular or circular, edge undulate or entire, liqufaction spreading.
Agar Colonies. 30^{o}C for 2 days. Growth rapid, irregular or rhizoid, smooth, pulvinate or umbonate, fimbriate.	30^{o}C for 2 days. Growth rapid, irregular or rhizoid, smooth or rough, umbonate or umbicilate, fimbriate.
Nutrient Broth. 30^{o}C for 2 days. Light pellicle, slight clouding, no sediment.	30^{o}C for 2 days. Light pellicle, slight clouding, no sediment.

Prosp	W3

Temperature relations (from growth on agar slants).

Prosp	W3
Optimum 30°C. Maximum between 49°- 56°C. Minimum " 5° - 20°C.	Optimum 30°C. Maximum between 49°-56°C. Minimum " 5° 20°C.

Relation to Reaction of Medium (Broth with HCl or NaOH)

Prosp	W3
Inhibited 4·2. Retarded about 9·8. Optimum pH. Between 9·5 and 5·8	Limits of pH for growth above 9·8 to 4·2. Optimum pH. Between 9·5 and 5·4.

Production of Indole (from tryptophane broth).

Prosp	W3
30°C for 2 and 5 days No indole.	30°C for 2 and 5 days. No indole.

Nitrate Reduction (from nitrate broth).

Prosp	W3
At 30°C for 1,2,4,7,and 10 days. No nitrites or gas formed.	At 30°C for 1,2,4,7,& 10day No nitrites or gas formed.

Milk (with litmus and brom. cresol purple as indicators also plain).

Prosp	W3
At 37°C for 10 days. Rennet curd in 4 days. Peptonisation begins in 4 days and continues slowly.	At 37°C for 10 days. Rennet curd in 7 days. Peptonisation begins in 7 days. Reduction of litmus begins in 12 days.

Hydrolysis of Starch (from starch agar plates)

Prosp	W3
At 30°C for 5 days. No hydrolysis.	At 30°C for 5 days. No hydrolysis.

Fermentation of Carbohydrates - glucose,lactose & sucrose broth with Brom. Cresol. purple as indicator.

Prosp	W3
At 30°C for 25 days Acid appears in glucose and sucrose in 2 days, in lactose in 25 days. No gas formed.	At 30°C for 25 days. Acid in glucose in 1 day,in sucrose in 2 days. None in lactose. No gas formed.

Chromogenesis.

Prosp	W3
On agar. Creamy-white.	**On agar.** Creamy-white.
On potato. Creamy, becoming yellow on second day.	**On potato.** Creamy-grey.

W_1	W_4
Isolated by Waters from Pink Rot	Isolated by writer from Pink Rot
Short rods arranged in singles and pairs. Size of majority 1·5μ x ·7μ Ends rounded.	Short rods arranged in singles and pairs. Size of majority 1·8μ x ·7μ Ends rounded.
Sporangia.	
Present of same size and form as vegetative cells. Staining with difficulty. Found sparingly in agar stroke cultures, incubated at 30°C for 4 days.	Present of same size and form as vegetative cells. Staining with difficulty. Found sparingly in agar cultures 4-7 days old.
Spores	
Central to excentric, elipsoid.	Central to excentric, elipsoid.
Motility	
Maximum motility in broth 10-18 hours. Film from 17 hour agar slope. stained Gray's method showed about 16 peritrichate flagella.	Max. motility in broth 10-18 hou Film from 17 hour agar slope. stained Gray's method showed about 16 peritrichate flagella
Gram positive becoming negative on 4th day.	Gram positive - negative on 3rd day.
Agar Stroke	
30°C 2 days Growth moderate, spreading, flat, glistening, smooth, irridescent, butyrous.	30°C 2 days. Growth moderate, spreading, flat, glistening, smooth, opaque to translucent, butyrous.
Gelatine Stab.	
At 20°C Liquefaction slow, crateriform begins in 2 days.	At 20°C. Liquefaction slow, crateriform begins in 2 days.
Nutrient Broth.	
30°C for 4 days Surface growth none, moderate persistent clouding, no sediment.	30°C Pellicle appears in 2 days. Clouding moderate - gone in 3 days. No sediment.

W_1	W_4

Agar Colonies

W_1	W_4
30°C for 1 day Growth rapid, irregular or rhizoid, smooth, effuse, fimbriate. Internal structure - amorphous.	30°C for 2 days. Growth slow, irregular, smooth, convex, undulate. Finely or coarsely granular.

Gelatine Colonies

W_1	W_4
20°C 4 days Growth slow, colonies circular or irregular. Edge undulate. Liquefaction spreading.	20°C 4 days. Growth slow, colonies circular or irregular. Edge entire or undulate. Liquefaction spreading.

Temperature Relations (from agar strokes)

W_1	W_4
Optimum 30°C. Maximum between 49°& 54°C Minimum " 5° & 20°C.	Optimum 30°C. Max. between 37°& 45°C. Min. " 5° & 20°C.

Relation to Reaction of Medium (from broth with HCl or NaOH).

W_1	W_4
Optimum pH between 9·5 & 5·6 Limits of growth from above pH 9·8 to 4·2.	Optimum pH between 9·5 & 5·4. Limits of pH for growth from above 9·8 to 4·6.

Production of Indole (from Tryptophane broth).

W_1	W_4
At 30°C for 2 & 5 days Indole absent.	At 30°C for 2 & 5 days. Indole absent.

Nitrate Reduction (from nitrate broth)

W_1	W_4
At 30 C for 1,2,4,7 & 10 days. No nitrites or gas formed.	At 30 C for 1,2,4,7,&10 days. No nitrites or gas formed

Milk (with litmus & Brom.Cresol.Purple indicator)

W_1	W_4
At 37 C for 10 days Rennet curd in 7 days. No peptonisation. Reduction of litmus begins in 6 days.	At 37 C for 10 days. Rennet curd in 10 days. Peptonisation slight in 10 days. Reduction of litmus begins in 9 days.

Starch (from stroke cultures on starch-agar plates)

W_1	W_4
No hydrolysis.	No hydrolysis.

Fermentation (glucose, lactose & sucrose broth with brom.cresol - purple as indicator) 30°C for 25 days.

W_1	W_4
Acid but no gas in glucose, lactose & sucrose. Acid appears in glucose & sucrose in 2 days. " " " lactose " 14 days.	Acid but no gas in glucose & sucrose. No appearance of acid in lactose after 25 days.

Chromogenesis

W_1	W_4
On potato creamy becoming yellow.	**On potato** creamy.

F_1	F_2
Isolated from platings of foot rot material.	Isolated from white, crumbling material from healthy foot of sheep.
Long rods. Arranged in singles, pairs and chains - but mostly chains. Size of majority 2·8μx 1·1μ (from agar slopes pH 7·0 at 30° for 20 hours).	Short rods. Arranged in singles, pairs and chains. Limits of length 1·3μ- 2·4μ Size of majority 1·9μ x ·6μ (from agar slopes pH 7·0 at 30° for 1 day) Variable - in older cultures chains of short and fatter cell appear.
Sporangia.	
In 24 hours almost every cell is a sporangium. Same size and shape as vegetative cells. Stain deeply with grams stain, leaving spore colorless.	Difficult to stain. Plentiful in 4 day agar cultures. Same shape as vegetative cells.
Spores.	
Eccentric elipsoid. 1·5μx 1μ	Central, elipsoid.
Motility.	
Maximum motility in broth 8-13 hours. Films from 7 hours agar slopes stained with Gray's flagellum stain. About 16 peritrichate flagella.	Maximum motility in broth 13-18 hours. Films from 17 hour agar slope stained with Gray's flagellum stain. Peritrichate flagella.
Gram positive.	Gram positive, becoming partly negative on 3rd day.
Agar Stroke. (from agar slopes at 30°C.)	
1 day.	2 days.
Growth moderate, spreading, flat, dull, rugose, opaque, white or yellowish, butyrous.	Growth moderate, spreading, flat and convex, dull, rugose, opaque, viscid.
Gelatine stab at 20°C.	
Liquefaction crateriform. Begins in 1 day, complete in 2 days.	Line of puncture, villous. Liquefaction crateriform begins in 6 days. Slow.

	F_1	F_2
Nutrient Broth at 30°C.	2 days Ring pellicle, moderate clouding, abundant flaky sediment.	2 days. Membranous pellicle, with drops of moisture above it, no clouding, no sediment.
Agar Colonies at 30°C for 2 days.	Growth rapid, filamentous, irregular, surface rough, convex. Edge filamentous and curled. Internal structure curled.	Growth slow, rhizoid, surface rough. Elevation, flat and convex to pulvinate. Edge filamentous and undulate. Internal structure filamento[us] and granular.
Gelatine Colonies at 20°C for 3 days.	Liquefaction rapid, complete in 3 days.	Growth slow, circular or irregular. Edge entire or undulate, liquefaction spreading. Internal structure filamentous.
Temperature Relations (from agar slopes)	Optimum temperature 30°C. Maximum for growth between 37°C - 45°C. Minimum for growth between 5°C - 20°C.	Optimum 37°C. Maximum for growth between 49°C - 56°C. Minimum for growth between 5°C - 20°C.
Relation to Reaction of Medium (from broth with HCl or NaOH at 30°C)	Retarded but not inhibited at pH of 9·8, inhibited at 4·2 Maximum pH for growth between 9·3 and 5·4.	Retarded but not inhibited at pH 9·8. Inhibited at 4·2. Maximum pH for growth between 9·3 and 5·4.
Production of Indole (from trytophane broth at 30° for 2 - 5day[s])	No indole	No indole.
Nitrate Reduction (from nitrate broth and agar slants at 30°C)	Nitrites from nitrate broth and agar in 1,2,4,7 & 10 days. No gas.	Nitrites produced from nitra[te] broth and agar in 4,7, & 10 days. No nitrites in 1 or 2 days, weakly positive reaction for nitrites on 3rd day.

F_1	F_2
Milk (Plain and with Brom.Cresol.Purple and litmus as indicator)	
At 37°C for 15 days. Rennet curd in 1 day Peptonisation begins in 1 day. Proceeds rapidly, complete in 12 days. Litmus not reduced.	At 37°C for 15 days. Rennet curd in 4 days. Peptonisation begins in 4 days. Reduction of litmus begins in 3 days. A narrow decolorized zone preceeds digestion.
Hydrolysis of Starch (from Starch agar plates at 30°C)	
Tested by alcoholic iodine solution. In 3 days hydrolysis little beyond growth.	In 3 days hydrolysis extending 1 cm. beyond growt
Fermentation (from glucose, sucrose & lactose broth, with Brom.Cresol.Purple).	
At 30°C for 25 days. Acid in glucose & sucrose in 1 day. Alkali appears in glucose and sucrose in 25 days. No gas.	At 37°C for 25 days. Acid in glucose & sucrose in 1 day. In 25 days glucose and sucrose tubes alkaline. No gas.
Chromogenesis .	
On Agar . White or cream.	On Serum. Crimson and pink abundant growth.
On potato. White.	On potato. Cream and pink or red growth.
	On Agar. Dull white or cream.

W2 ISOLATED BY WATERS FROM PINK ROT.

Vegetative Cells.

Short rods and long rods arranged singly and in pairs and chains.

Limits of length $6{\cdot}6\mu$ - 2μ.

Size of majority $3{\cdot}3\mu \times 1{\cdot}5\mu$.

Ends rounded. Cells variable in size and form.

(from agar slopes pH 7·0 incubated 30°C for 1 day)

Sporangia.

Found sparingly in 2 day agar cultures. Sporangium in form of drumstick. Easily stained. Small terminal spore remains unstained.

Motility.

Maximum motility in broth about 13 hours. Never a large percentage motile.

Films from 17 hours agar slopes showed peritrichate flagella.

Gray's Method used.

Irregular forms.

On 1 day agar slopes at 30°C.

Rods may be slightly twisted or swollen at both ends.

Agar Stroke.

At 30°C for 1 day.

Growth moderate, spreading, flat, glistening, surface smooth, opaque, butyrous.

Gelatine Stab at 20°C.

Villous growth near surface in 4 weeks, liquefaction just begins in 6 weeks.

Nutrient Broth at 30°C for 1 day.

Fine ring pellicles forming festoons. Slight clouding. Abundant compact sediment appears in 2 days.

Agar Colonies, at 30°C for 2 days.

Rhizoid, smooth, effuse or convex, edge fimbriate. Internal structure finely granular.

Gelatine Colonies 20°C for 5 weeks.

Growth slow. Very small circular or irregular colonies, raised but later crateriform. Edge entire or undulate. Liquefaction saucer-like. Internal structure, finely granular.

Temperature Relations (on agar slopes)

Optimum temperature for growth 30 C.
Maximum temperature for growth between 37° - 45° C.
Minimum temperature for growth between 5° - 20°C.

Relation to Reaction of Medium (In broth with HCl or NaOH).

Optimum pH for growth between 9·5 - 5·8.
Growth retarded about pH 9·8.
Inhibited at pH 5·4 .

Production of Indole (from tryptophane broth at 30°C.
No indole in 2 or 5 days.

Milk (Plain or with Brom.cresol.purple and litmus as indication)
At 30°C.
No change in 10 days.
Slight alkalinity to Brom.cresol.purple in 12 days.
No curd and no peptonisation.

W_2 Contd.

Reduction of Nitrates (in nitrate broth at 30°C.)

No nitrites or gas in 1,2,4,7, or 10 days.

Hydrolysis of Starch (from starch agar plates)

No hydrolysis.

Fermentation (glucose, sucrose and lactose broth and brom.cresol. purple).

At 30°C for 25 days.

No production of acid or gas in 25 days.

Chromogenesis.

On agar. Whitish growth.

On potato. Creamy-grey.

S U M M A R Y.

Three organisms (two from pink rot and one from crumbling hoof) have been isolated and have been shown to ret wool in water. The time for retting of wool scoured in warm water and ether, sterilized by discontinuous steaming, inoculated from a turbid broth culture in 5 c.c. of sterile distilled water and incubated at 30 C, was about 6 weeks. Fibres retted in water were not discoloured. The two organisms isolated by writer from pink rot and two isolated by Waters from the same source, were found to be much alike in their morphological and physiological characters and all were good wool retters.

Their characteristics may be summarized thus :

Short rods between ·5 and 1μ, length more than 2 diameters, chains absent, gram positive, flagella peritrichiate. Endospores central to excentric, elipsoid or cylindrical, diameter less than that of vegetative cells. Geltaine liquefie nitrates not reduced, starch not hydrolised, acid from glucose and sucrose. In milk rennet curd formed. Agar strokes - growth moderate, lustre glistening, surface smooth. Agar colonie rhizoid.

The retting capacities of Bacillus megatherium were tested under similar conditions to those just described for the pink rot organisms. B.megatherium was reported by Trotman and Sutton 1924, as retting woollen fabrics and by Burgess 1924 as retting commercially scoured wool. It was found to ret wool which had not been processed but merely scoured in warm water and ethe in about 6 weeks. B.megatherium was markedly slower in its actions upon wool in broth than the bacteria isolated from pink rot but there was no significant difference in the time taken for B.megatherium and the pink rot organisms to ret wool in water.

B.mycoides and B.vulgatus did not ret wool so fast or thoroughly as B.megatherium or the pink rot organisms.

The bacteria isolated from wool and able to ret it were able to rot autoclaved hoof in the presence of broth.

Organisms isolated from foot rot parings and from crumbling material from healthy hoofs were able to ret autoclave wool and hoof in broth cultures.

My experiments indicate that retting organisms may be present in sound unaffected fleeces, in the soil, in parings from feet of sheep suffering from foot rot, and in crumbling material from the soles of healthy hoofs.

Locks of sound unsterile wool suspended in humid atmosphere and incubated at 30 C rot much more quickly at the weathered tips (about 2 inches) of the locks than at the butts (first 2 inches or so of lock).