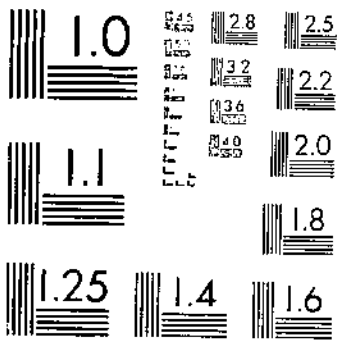
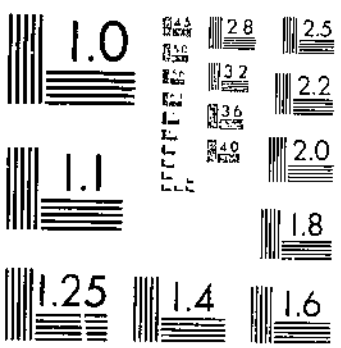
START



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963-A



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963-A

Technical Bulletin No. 588 September 1937

UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

THE STERILIZATION OF WOOL AND ITS EFFECT ON PHYSICAL AND CHEMICAL PROPERTIES OF A WOOL FABRIC[1]

By HARRY HUMFELD, *associate bacteriologist, Division of Soil Microbiology, Bureau of Plant Industry;* RUTH E. ELMQUIST, *associate textile chemist;* and JAMES B. KETTERING, *junior chemist, Textiles and Clothing Division, Bureau of Home Economics*[2]

CONTENTS

INTRODUCTION

From the time wool grows on the sheep, on through the stages of manufacture and wear, the fibers are subject continually to contamination with micro-organisms. Included in this micro-organic flora may be the pathogenic organisms responsible for disease. There may also be other groups less dangerous to health but capable of multiplying rapidly under favorable conditions and causing the wool fibers to be stained with mildew or to lose strength and deteriorate in other ways. The utilization of wool therefore may be seriously affected by the action of micro-organisms.

Oftentimes wool must be sterilized to free it of pathogenic organisms, and also of the nonpathogens which damage it as already indicated. Also in bacteriological studies of the effect of these micro-organisms on the fibers, it is essential that the wool be sterilized.

Many of both these groups of organisms have a sporulating stage, during which they are very resistant to the usual means of sterilization. It is generally believed that these spores are not killed until

[1] Received for publication May 11, 1937.

[2] This is the initial publication of a study of the deterioration of wool fabrics by micro-organisms which has been undertaken cooperatively by the Textiles and Clothing Division of the Bureau of Home Economics and by the Division of Soil Microbiology of the Bureau of Plant Industry. The Bureau of Home Economics requested the cooperation of the Bureau of Plant Industry on this project since Charles Thom and Harry Humfeld of the latter Bureau had previously published a study on the effect of certain micro-organisms on a cotton fabric and were familiar with the technique involved in such work. Also, *Bacillus mesentericus* and *B. subtilis*, the organisms active in the deterioration of wool, are those occurring in soils.

their protoplasm is coagulated. Since wool is a protein and since the protoplasm of the spores is also largely protein in nature, it becomes evident that the destruction of the spores without damaging the wool is by no means an easy problem.

The investigation reported here resolved itself into testing methods that might be efficient for killing the spores contaminating the wool and then determining the effects of the efficient methods on the physical and chemical properties of the fibers. Obviously the method selected for use in subsequent bacteriological studies will be the one that effectively sterilized the wool with the least physical and chemical changes.

LITERATURE REVIEW

A survey of the literature indicated that relatively little work has been done on methods of killing micro-organisms in the great variety of textile materials. The only quantitative results reported on the effect of sterilization on textiles are breaking-strength values. No quantitative data have been found on the effect of sterilizing treatments on wool.

The literature review presented here concerns itself with: Killing the spores of *Bacillus mesentericus;* sterilization of wool; and additional sterilizing methods which hitherto had not been used for wool.

An organism present on wool and one of the hardest to kill is *B. mesentericus.* Globig (*20*)[3] found that it was necessary to use a current of steam for 5½ to 6 hours to destroy the spores of this organism. When he increased the temperature to the range of 109° to 113° C., the spores were killed in 45 minutes; with a progressive rise in temperature the time was decreased, until at 130° they were destroyed instantly. He found that 1½ hours were required to kill the spores of *B. mesentericus* placed in a 1-percent solution of mercuric chloride, while 2 weeks in a 5-percent phenol solution proved ineffective. Coulthard (*10*) states that the spores survived hot air at 320° for 30 minutes. Many other investigations could be cited to indicate the resistance of *B. mesentericus* to adverse conditions.

The steaming of wool in distilled water for 20 minutes at 100° C. on each of 3 successive days is reported by Burgess (*7*) to produce sterility. He (*8*) stated that the filtered extract obtained from wool subjected to four successive 20-minute steamings in distilled water supported a copious fungal growth. This indicates that steaming caused considerable hydrolysis of wool.

Trotman and Sutton (*40*) also used intermittent steaming as a means of sterilizing wool. Their procedure differed from that of Burgess in that they added a small amount of nutrient broth to the water. The presence of small amounts of residual nutrient on the wool might make it more susceptible to attack by micro-organisms. However, sterility would probably be more easily attained in their treatment, because the spores would be more likely to germinate in the periods between steamings.

Prindle (*32*) used the method of Trotman and Sutton (*40*) for sterilizing skeins of wool yarns. He determined the effect of bacteria on the strength of the yarn by breaking it between his fingers.

[3] Italic numbers in parentheses refer to Literature Cited, p. 24.

Wool blankets and uniforms in Army cantonments and base hospitals were sterilized by Fulton and Staniford (*17*) by subjecting them to steam pressure at 12 to 15 pounds from 10 to 30 minutes. They found that the resultant deterioration was so pronounced that it easily could be observed visually.

Wool imported from Persia, Syria, and China is often heavily infected with anthrax. Macdonald (*26*) found that almost complete destruction of the anthrax bacteria could be accomplished by immersing wool in a 10-percent formaldehyde solution and passing it over a heated drying cylinder. Wool treated with 8-percent formalin was observed by Burgess (*7*) to remain sterile; lower concentrations of formalin permitted growth. When he (*8*) suspended wool inoculated with *B. mesentericus* over the vapors of a 4-percent formalin solution, it was still sound after an interval of 45 days' exposure. The English Government now compels all imported wool to be sterilized by a formaldehyde process.

Latzke (*25*) reported that ultraviolet light was more effective as a sterilizing agent on cotton, linen, and silk than on wool of similar interspace and considered that the size of the interspaces was important in the penetration of the light through a fabric.

According to Pratt (*31*) both fungal and bacterial stains are removed from woolen fabrics by treatment with potassium permanganate followed by oxalic acid.

Ceredi (*9*) observed that wool required 25 times its weight of a 5-percent solution of mercuric chloride to sterilize it when inoculated with tubercular material.

Some germicidal treatments which have been reported by investigators working on other substances may be useful for sterilizing wool. Schaffer and Tilley (*35*) found that germicidal soap made by adding orthophenylphenol to coconut-oil soap was efficient against *Staphylococcus aureus* even in the presence of milk and blood serum. Various phenylphenates are now commercially available, and their use as germicides appears promising.

The toxicity of mercuric salts according to Paul and Krönig (*30*) is proportional to their degree of dissociation. Buchanan and Fulmer (*6, p. 398*) report that iodine is highly toxic to micro-organisms and that 3.8 to 5 parts per million efficiently sterilizes water.

According to Coulthard and Sykes (*11*) the addition of alkali, acid, or amylmetacresol to methyl, ethyl, or isopropyl alcohol increased the germicidal effect of these alcohols.

Bechhold (*3*) found that tribrombetanaphthol is not only a good germicide for streptococci but practically odorless and not very poisonous. Tetrachlorethane was used by Ezekiel and Taubenhaus (*14*) to control growth of the cotton root rot organism, but this substance has a pronounced inhibitory effect on the cotton plant.

Xylene in the proportion of 500 to 1,000 parts per million in soil Ezekiel and Taubenhaus (*15*) reported to be effective in controlling cotton root rot and produced no inhibitory effects on cotton planted 16 days after treatment. Morel, Rochaix, and Mathais (*29*) studying the antiseptic power of xylene on colon bacilli found that only comparatively small amounts were required to check the growth of this organism, while Benians (*5*) observed that toluol, benzol, and xylol had no effect on spores but readily killed Gram-negative organisms.

BACTERIOLOGICAL PROCEDURE

METHOD FOR TESTING THE EFFECTIVENESS OF TREATMENTS FOR STERILIZING WOOL

A uniform procedure was adopted in order to evaluate the sterilizing methods correctly. The general technique used in this investigation for testing the effect of various methods was as follows:

An undyed, laundered, wool blanket fabric having a thickness of 0.159 inch and weighing 23.9 ounces per square yard was used. Blanket material was selected for the bacteriological tests, because it was considered as difficult to sterilize as any fabric that might be encountered. The thickness and degree of matting of the particular piece of blanket fabric chosen offered excellent protection for the micro-organisms. The material was saturated with an aqueous suspension of spores and cut into squares of approximately 1.5 centimeters. Five squares were used for each sterilizing treatment and five for each control.

Since any effective sterilizing treatment must kill all organisms that might be present, it was necessary to inoculate with one known to be extremely resistant to adverse conditions. *Bacillus mesentericus* was chosen as a representative sporulating form, and it also had been used by other investigators for purposes of inoculation. Moreover, this particular micro-organism has been described as destructive to wool fibers (*7, 8*). The strain of *B. mesentericus*[1] used for inoculation was a transfer of no. 726 of the American type culture collection, 1923.

The medium used throughout this investigation had the following composition: Beef extract, 3 grams; peptone, 10 grams; agar, 10 grams; and water, 1 liter. The pH was adjusted to 6.8 to 7.0 by adding normal sodium hydroxide solution. The medium was then sterilized at 15 pounds steam pressure for 30 minutes.

Cultures of *B. mesentericus* were spread over the surface of the sterile medium in Petri dishes and allowed to grow for several days at 28° C. An aqueous spore suspension of sufficient strength to give a milky appearance was made by washing the growth from the Petri dishes into a bottle of sterile water.

Wool squares were immersed in the milky suspension. When saturated they were removed and spread on paper. As soon as they were air-dry, they were ready to be used for the sterilizing treatments.

The various sterilizing treatments are described under their respective headings. Whenever these treatments were of a chemical nature, the wool squares were washed at least twice in sterile water at room temperature for 5 minutes or longer to rinse out the excess disinfectant prior to testing for the survival of spores.

Immediately after the sterilizing treatment, or treatment and washing, each square of wool was transferred by means of sterile forceps to the center of the agar medium in a Petri dish. The inoculated but untreated controls were plated out at the same time. In order to avoid mistaking a residual antiseptic effect for sterilization, the agar plates were tilted to allow the excess fluid on the

[1] Appreciation is expressed to N. R. Smith, of the Bureau of Plant Industry, for the culture of *B. mesentericus*.

fabric to run across the medium to the edge of the plate. If the toxic residue on the fabric had merely an antiseptic effect, any surviving organisms in this fluid, upon incubation, would multiply and produce a visible growth. Growth would be possible, since this fluid which had run to the edge of the plate would be beyond the zone of bacteriostasis.

Whenever the surviving spores grew only in the outer regions of the plate (fig. 1, *C* and *D*), the results were expressed as "antiseptic

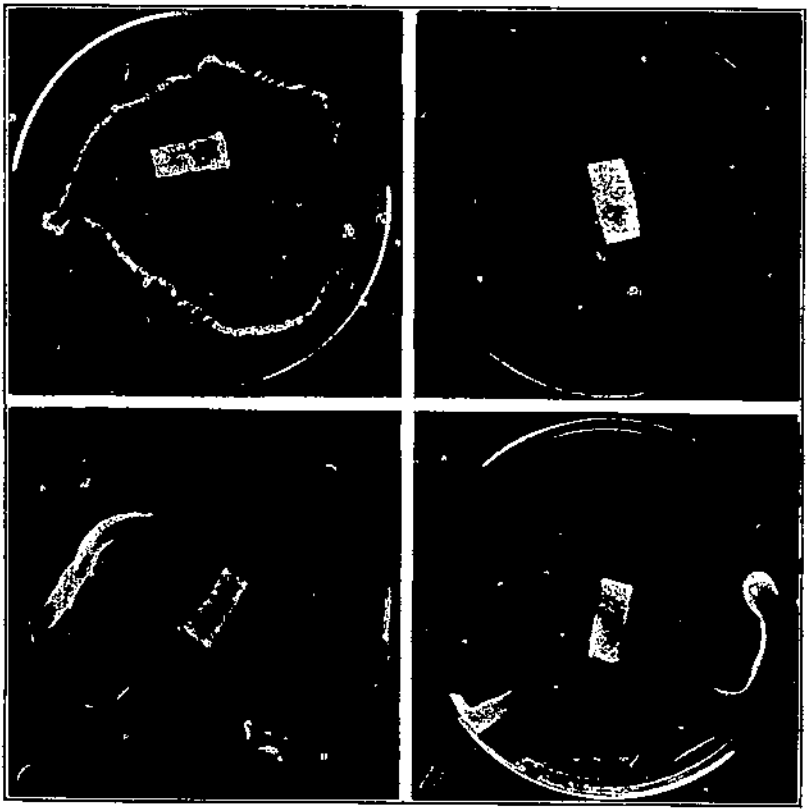


FIGURE 1.—*A*, Normal growth of *B. mesentericus* on fabric and agar; *B*, sterile fabric on agar; *C*, the inhibitory effect of sodium-2-chlor-4-orthophenylphenate on the growth of *B. mesentericus* on wool; *D*, the marked inhibitory effect of sodium-2-brom-4-orthophenylphenate on the growth of *B. mesentericus* on wool.

action without sterilization." Many of the tests for spore survival gave no growth whatever on plates which received the chemically treated and rinsed fabrics. In this case the results were recorded as "apparent sterilization." All the plates that produced this result were then reinoculated with fresh spore suspension in order to learn whether the failure of growth might be attributed to residual bacteriostatic substances in the rinsed wool. If the wool inhibited growth, the result was termed "apparent sterilization with bacteriostatic residue." In the cases (i. e., heat, alcohol, etc.) where the method left no bacteriostatic residues, the results were expressed as "sterilization" or "no sterilization."

It will be noted that the term "apparent sterilization" has been applied to all instances in which treatment with chemical agents was used and in which it was impossible to demonstrate the survival of spores, while the terms "antiseptic action", "growth inhibition", or "bacteriostatic residue" were used when the agent interfered with the growth of the test organism without actually sterilizing the fabric.

In this connection, it is obvious that any procedure which is to be employed in further studies on the effect of bacteria in the deterioration of wool must bring about sterilization without leaving bacteriostatic residues in the wool and must also cause a minimal change in the physical and chemical properties of the wool fabric.

EXPERIMENTAL RESULTS

The results of attempts to sterilize wool were classified in the following way:

Unsatisfactory:

Sterilization without bacteriostatic residue but with damage to the fabric.
Sterilization with bacteriostatic residue and with damage to the fabric.
No sterilization and no bacteriostatic residue.
No sterilization but bacteriostatic residue.

Satisfactory:

Sterilization, no bacteriostatic residue, and minimal damage to the wool as evidenced by physical and chemical tests.

STERILIZATION WITHOUT BACTERIOSTATIC RESIDUE BUT WITH DAMAGE TO THE FABRIC

INTERMITTENT STEAMING

The intermittent steaming method is based on the supposition that the bacterial spores germinate during the intervals between steamings; that is, although the spores are resistant to steam at 100° C., the vegetative cells are killed. Intermittent steaming was tested first since this method of sterilization has been used most frequently by investigators (*7*, *32*, *40*) studying the bacteriology of wool. A set of five wool squares was saturated with water, placed in a Petri dish, and steamed in an autoclave at 100° for 30 minutes on 3 successive days. Another set was similarly treated on 4 successive days. Two duplicate lots of dry samples were steamed at the same time.

Steaming of wet squares on 4 days was the only one of these procedures that gave sterility. The data obtained showed that three intermittent steamings did not kill all of the organisms. Although four steamings did give satisfactory results as far as sterility was concerned, tests for chemical and physical changes indicated progressive deterioration of the wool, so that by the time the material had been rendered sterile, marked changes had occurred. Therefore the method was discarded.

AUTOCLAVING

Although high temperatures are known to produce marked chemical and physical changes in wool, sterilization by means of autoclaving was included in this study because it is a convenient and cer-

tain means of killing micro-organisms. Wool squares were autoclaved in both the wet and the air-dry condition. They were placed in Petri dishes and the dishes were placed in the autoclave in layers not more than three deep. Tests were made using 5, 10, and 15 pounds steam pressure for 20 minutes, and using 15 pounds steam pressure for periods varying from 5 to 90 minutes on the wet squares and from 30 to 240 minutes on the dry.

Five- and ten-pound steam pressures were ineffective. At 15 pounds pressure in Petri dishes the wet and dry squares were sterile after 30 minutes. The rapidity of the heat penetration was found to be important. If the dry samples were placed in 16-ounce square, screw-top bottles, a much longer time was needed for sterilization than in Petri dishes. From a bacteriological standpoint, sterilization by autoclaving was entirely satisfactory. However, the physical and chemical changes produced in the fabric disqualified this method.

Harrison (*21*) states that wool boiled in 10-percent acetic acid loses less strength than wool boiled in water. Tests were made by moistening the wool squares with 10-percent acetic acid, drying, and then autoclaving at 15 pounds steam pressure for 10, 20, 30, and 60 minutes. The acetic acid was then neutralized by washing in a sterile sodium carbonate solution and the residual salts were removed by two 10-minute rinses in sterile water. A minimum time of 30 minutes was required for complete sterilization, which is apparently the same as that required when the wet samples were autoclaved. Subsequent tests showed that wool treated with an acetic acid solution lost more strength than when saturated with water.

AUTOCLAVING IN MINERAL OIL

A study of the chemical and physical changes occurring in a wool fabric autoclaved wet showed clearly the damaging effect of moisture on wool during heating. It seemed probable that wool might be less damaged during sterilization if immersed in some rather inactive liquid such as mineral oil. Wool squares were consequently placed in mineral oil and heated in the autoclave at 15 pounds steam pressure for 5, 10, 15, and 20 minutes. The mineral oil was removed by washing first in chloroform, then in ethyl alcohol, and finally in sterile water. All of the squares showed abundant growth upon incubation.

Wool squares were autoclaved in mineral oil for 20 minutes at 15 pounds steam pressure. After removal from the autoclave the flask containing the wool fabric and the mineral oil was heated until the temperature of the oil reached 200° C. Although it was found that the organisms had been killed by this treatment, the wool material showed definite evidence of deterioration.

STERILIZATION WITH BACTERIOSTATIC RESIDUE AND WITH DAMAGE TO THE FABRIC

FORMALDEHYDE

The wool squares were immersed in solutions of formaldehyde varying from 2½ to 10 percent for 30 minutes at room temperature. Only those pieces treated with solutions that were 8 percent or

stronger were found to be sterile when plated out. A 2½-percent solution of formaldehyde at room temperature gave sterility after a 60-minute treatment but not after 40 minutes. A 10-percent solution was tried for 10, 20, and 30 minutes. The 30-minute treatment produced sterility. Wool squares were exposed to concentrated formalin vapors both at room temperature and at 70° C. for 10, 20, 30, 45, 60, and 120 minutes. Only the exposure for 120 minutes at 70° was effective.

In the cases of incomplete sterilization with formaldehyde, the growth of the bacteria occurred on the agar only at a distance from the fabric. The extent of the clear zone left around the fabric was apparently determined by the amount of formaldehyde left after the washing procedure. Formaldehyde was very definitely an inhibitor of bacterial growth in concentrations much lower than those required for sterilization. Inhibition of growth was also produced when the treatment was effective.

MERCURIC SALTS

Since mercuric chloride has been used repeatedly for disinfection and sterilization, wool squares were immersed in 0.1-, 0.2-, 0.3-, 0.4-, and 0.5-percent solutions of mercuric chloride for 30 minutes at room temperature. The results indicated that under these conditions the minimum lethal concentration was 0.3 percent.

Another series of tests were made using 0.4-percent concentrations for 4, 6, 8, 10, 20, and 30 minutes at room temperature followed by two 10-minute washes with sterile water. The minimum length of time required for sterilization was found to be 10 minutes.

Treatments with 0.4-percent solutions of mercuric nitrate, acetate, and cyanide for 30 minutes at room temperature were next tried followed by two 5-minute washes with sterile water. The number of washes was increased to three and hot water was used to facilitate the more complete removal of the mercuric salts, as marked inhibition was evident in the previous tests. All of the salts used at this concentration and for the length of time stated produced complete sterilization accompanied, however, by marked bacteriostatic residue, as indicated when subsequent reinoculations with *B. mesentericus* were made. Mercuric iodide proved to be too insoluble to be used for sterilizing purposes.

Both formaldehyde and mercuric salts may be used for the sterilization of wool fabrics but subsequent bacteriostasis makes these methods useless for bacteriological studies.

NO STERILIZATION AND NO BACTERIOSTATIC RESIDUE

ULTRAVIOLET LIGHT

As ultraviolet light has been used (*18, 25*), to kill micro-organisms, it seemed that it might prove of value in the present work. Wool squares were exposed in an opened Petri dish to the rays of a mercury vapor lamp using 5 amperes at 110 volts alternating current. Three sets of squares were exposed at a distance of 12 inches from the arc as follows: (1) 15 minutes on each side; (2) 15 minutes on each side with the dish tilted at a 45° angle, first in one and then in the opposite direction to permit better penetration of the rays

under the individual fibers; and (3) 30 minutes on each side with the dish tilted at a 45° angle in four directions. After all these ultraviolet treatments good growth occurred on the test medium in less than 24 hours. The ineffectiveness of ultraviolet light was no doubt due to the protection given the bacteria by the wool fibers. Apparently the rays were intercepted by the fibers before reaching the micro-organisms.

POTASSIUM PERMANGANATE

Potassium permanganate has been used for removing mildew stains from fabrics (*31*); but no observations were reported concerning its germicidal value. Therefore a series of permanganate solutions varying in concentration from extremely dilute to saturated was tried. None produced sterility. The chemical effect of the potassium permanganate was so severe in the higher concentration that the wool fabric was completely disintegrated.

IODINE

Although iodine is a good disinfectant (*6*), its use for sterilizing wool had not been investigated. Squares treated with a saturated aqueous solution of iodine followed by neutralization with sodium thiosulphate showed growth in 4 days. In addition the iodine stained the wool to such an extent that this in itself would be objectionable.

ALCOHOL

Ethyl alcohol is often used as a disinfecting agent. It is readily available as 95-percent alcohol, but is considered to be a more effective sterilizing agent if diluted to 60-percent strength. Wool squares were immersed in solutions of 60- and 95-percent ethyl alcohol at room temperature for 30 minutes. Since a heavy growth appeared on the sample within 24 hours, the experiments were repeated raising the temperature to 70° C., a few degrees below the boiling point of alcohol, in an effort to increase its effectiveness. As at room temperature, growth again occurred within a day.

These findings agree with the statement of Rosenau (*34, p. 1423*) that although 50-percent ethyl alcohol kills most vegetative forms of bacteria quite readily, many spores are unaffected by it at any concentration.

Since the use of ethyl alcohol was unsuccessful, the effect of some of the alcohols with higher boiling points was studied. The squares of wool were boiled in propyl alcohol for one-half, 1, 2, and 4 hours. Other sets were autoclaved at 15 pounds steam pressure for similar periods of time in a mixture of a solution of 80-percent propyl and 20-percent butyl alcohol (boiling point 97° C.). Additional sets were autoclaved in butyl alcohol (boiling point 118°) for periods of 1, 2, 3, 4, and 6 hours. Each of the above treatments was followed by three 10-minute washes in sterile water. Boiling amyl alcohol was finally tried for 3 hours followed by one 10-minute alcohol wash and two 10-minute washes with sterile water.

The rather surprising results indicated that *B. mesentericus* was resistant to alcohol at high temperatures for a considerable length of time, as in no case, even with the longest time and the highest

temperatures, did all of the five replicate squares of fabric remain sterile. In general, no loss in breaking strength resulted from these alcohol treatments.

According to Holmes (*23*), Persoz found that wool saturated with 10-percent glycerol can be exposed to temperatures as high as 140° C. without harm. However, in the experiments reported here, wool squares were autoclaved in 10-percent glycerol at 15 pounds steam pressure for 1 and 2 hours, at 20 pounds for 2 hours, and at 30 pounds for 1 hour. They were so badly browned and their strength so impaired that they were not even plated out. In an effort to avoid hydrolysis of wool during the heating, undiluted glycerol was used. Similar results were obtained.

TRIBROMBETANAPHTHOL

Tribrombetanaphthol has been found to be a very efficient germicide in certain instances (*3*). However, samples of the woolen fabric given a 30-minute treatment with saturated tribrombetanaphthol solution showed abundant growth at the end of 24 hours. It may be concluded, therefore, that it is entirely ineffective, at least on the spores of *B. mesentericus*.

TETRACHLORETHANE

A definite germicidal effect has been obtained with tetrachlorethane (*15*). Wool was treated by immersing the samples in the concentrated halogen compound for 30 minutes, then air drying and plating them under sterile conditions. Growth resulted after 4 days. It was apparent, therefore, that considerable percentage of the organisms were killed, but that a sufficient number survived to show visible growth after a number of days incubation.

NO STERILIZATION BUT BACTERIOSTATIC RESIDUE

SODIUM PHENYLPHENATES

The value of various sodium salts of phenol derivatives for sterilizing wool fabrics was studied, because investigators in the Bureau of Animal Industry (*37*) had found them effective in controlling disease organisms. The wool squares were immersed in a 1-percent sodium orthophenylphenate at 30° and 40° C.; 1 percent sodium chlororthophenylphenate at 30° and 40°, and 2 and 3 percent at 40°; 1-percent sodium-2-chlor-4-orthophenylphenate at 30° and 40°; and 2 and 3 percent sodium-2-brom-4-orthophenylphenate at 40°. The time of each treatment was 30 minutes.

None of these processes gave sterility. However, the phenylphenates without exception had pronounced bacteriostatic effects which were indicated by the inhibition of growth on the agar even at a considerable distance from the fabric. Moreover, the amounts of the phenates left in the squares after two successive transfers to 200 cubic centimeters of sterile water must have been exceedingly small, especially since the original solutions were very dilute.

Figure 1, *C* and *D*, shows the effect of the diffusion of traces of sodium-2-chlor-4-orthophenylphenate and sodium-2-brom-4-orthophenylphenate, respectively, into the culture medium. In both *C* and

D there is a clear zone around each square of wool. The extent of the diffusion which determines the limits of growth of the bacteria and therefore the clear zone is greater for figure 1, *D*, than for *C*.

Normal growth of *B. mesentericus* on the fabric and agar of the controls is shown in figure 1, *A*. The plates had a similar appearance when the particular sterilizing treatment tested was unsuccessful, or when sterilization without inhibition was accomplished and the sterile fabric reinoculated. Figure 1, *B*, illustrates the appearance of a square of sterile fabric on the medium.

STERILIZATION, NO BACTERIOSTATIC RESIDUE, AND MINIMAL DAMAGE TO THE WOOL

HEATING IN XYLENE

Xylene is an organic liquid which has antiseptic properties and which also might protect wool from the damaging effects of heat. The xylenes boil at from 138° to 144° C. and would not be heated to their boiling points at steam pressures as high as 30 pounds (134.5°). It seemed reasonable, therefore, that the use of xylene as an antiseptic plus the use of heat as a sterilizing agent might prove more effective than either alone.

Wool squares were placed in xylene and autoclaved for ½, 1, 2, 3, and 4 hours at 30 pounds steam pressure. Another set of squares were autoclaved for the same periods at 15 pounds steam pressure. The xylene was removed by one alcohol rinse and two 10-minute rinses in sterile water. At 30 pounds all time limits of 1 hour or more gave complete sterility while at 15 pounds (121° C.), at least 2 hours were necessary.

In order to determine the lowest temperature practical for sterilizing wool in xylene, the squares were immersed in this solvent at room temperature and samples were removed and plated out after different periods, varying from 1 day to 4 weeks. Sterility was not obtained even after the longest time. Wool was immersed in xylene at 100° C. for 4, 6, 12, and 24 hours. After 12 hours all organisms had been killed.

Heating at 100° C. or autoclaving in xylene, therefore, gave entirely satisfactory results from a bacteriological standpoint. Reinoculation with *B. mesentericus* resulted in abundant growth and preliminary tests indicated that the changes in the wool fabric, if any, were negligible.

HEATING IN STODDARD SOLVENT

The experiments with xylene were duplicated with Stoddard solvent to learn whether solvents such as those employed in the dry-cleaning industry could be used as well as xylene for the sterilization of wool. Stoddard solvent, a petroleum fraction distilling between 145° and 210° C. and usually referred to as mineral spirits, is the cleaning fluid used most extensively in the dry-cleaning of textile fabrics. The results with this solvent were found to be very similar to those obtained with xylene. Heating at 100° for 12 hours, at 121° for 2 hours, or at 134.5° for 1 hour gave sterility. Reinoculation indicated that no toxic residue remained.

HEATING IN TETRACHLORETHYLENE

Tetrachlorethylene is employed to some extent as a solvent in the dry-cleaning industry. Wool squares were immersed in it under the conditions outlined for xylene. One hour at 134.5° C. and 2 hours at 121° produced sterility. However, at 100° it was necessary to treat the wool for 24 hours before sterility was obtained. Subsequent inoculation with *B. mesentericus* proved that any residue which might remain in the wool was not toxic.

PHYSICAL AND CHEMICAL PROCEDURE

A satisfactory method of sterilizing wool for bacteriological studies not only must give sterility unaccompanied by subsequent inhibition but also must allow the material to retain as nearly as possible its original physical and chemical characteristics. The treatments selected for physical and chemical study are summarized in table 1.

TABLE 1.—*Sterilizing treatments given the serge fabric*

Treatment no.	Treatment procedure	Effect of treatment
1	Autoclaving in xylene for 1 hour at 30 pounds steam pressure	Sterilization.
2	Autoclaving in xylene for 2 hours at 15 pounds steam pressure	Do.
3	Autoclaving dry for ½ hour at 15 pounds steam pressure	Do.
4	Autoclaving dry for 1 hour at 15 pounds steam pressure	Do.
5	Intermittent steaming for ½ hour at 100° C. on 3 successive days	No sterilization.
6	Intermittent steaming for ½ hour at 100° on 4 successive days	Sterilization.
7	Autoclaving wet for ½ hour at 15 pounds steam pressure	Do.
8	Treatment in a 2½-percent formaldehyde solution for 1 hour at room temperature.	Sterilization, with inhibition.
9	Treatment in a 10-percent formaldehyde solution for 1 hour at room temperature.	Do.
10	Treatment in a 0.4 percent mercuric chloride solution for 10 minutes at room temperature.	Do.
11	Treatment in a 0.4-percent mercuric chloride solution for 30 minutes at room temperature.	Do.

A serge fabric was used in these treatments instead of the blanket material. All of these processes, except the 3-day intermittent steaming, the formaldehyde, and the mercuric chloride, produced sterility without bacteriostatic residue. The 3-day steaming was included because it has been used repeatedly by investigators studying the bacteriology of wool and the formaldehyde and mercuric chloride treatments because they produced sterility even though it was accompanied by subsequent bacteriostasis. The two latter treatments may be valuable for purposes other than bacteriological studies.

Physical and chemical results are grouped by tests measuring deterioration rather than by sterilizing treatments. The physical tests used were: Strength index, weight, thickness, and flexural properties. Chemical damage was estimated by determining the changes produced in the percentage of sulphur and nitrogen, in methylene blue absorption, and by scale breakage.

MATERIAL AND METHODS

The fabric used for determining the physical and chemical changes caused by various sterilizing treatments was a clear finished, bleached, all-wool, 2 up and 2 down even-twill serge. Although the thick,

felted blanket fabric was an excellent textile for the bacteriological tests, the serge was a better choice for the physical and chemical analyses. Breaking-strength strips are difficult to prepare from the blanket material, because of its napping and excessive felting. Moreover its low breaking strength and thread count would cause large errors in the measurement of changes in these properties.

For all tests except flexural work, flexural resilience, and breaking strength, samples were prepared by cutting 6-inch squares of the serge through the middle in two directions to form four 3-inch squares. Of the squares thus formed, the upper right hand and the lower left hand were given a sterilizing treatment and used as a sample while the other two afforded a control. The variability of a fabric makes it desirable to analyze a control from the immediate vicinity of the sample and of the same warp and filling.

Six 6-inch squares gave sufficient material for both samples and controls for each sterilizing treatment, since for some of the tests it was unnecessary to use all of the fabric in the four smaller pieces. For each separate determination, material was taken from both the upper right-hand and lower left-hand squares of an original 6-inch piece and also from both corresponding control sections. The sampling for the flexural work and flexural resilience differed from the above only in the size of the original piece cut for analysis.

Breaking-strength determinations for each treatment were made on a set of five warp strips cut from various parts of the fabric. A set of 10 warp strips similarly selected served as the average control for all of the treatments.

The sterilizing treatments given the serge fabric used for the physical and chemical measurements were the same as those given the blanket material for the bacteriological work. Additional rinses might have removed more completely any traces of the reagents remaining in the wool after the chemical treatments. If these traces had been eliminated, slightly different values might have been obtained for the physical and chemical measurements. However, it is not feasible to rinse material used for bacteriological work any great number of times, as the danger of contamination increases with the number of rinses.

The sample dimensions for each physical measurement and the weight for each chemical test are given under the description of methods. The number of determinations is reported in tables 2, 3, and 4. All the measurements were made on wool thoroughly conditioned at 70° F. and 65-percent relative humidity, but results of the chemical analyses were calculated on the basis of the oven-dry weight.

Strips cut 1¼ inches wide and 6 inches long were raveled to exactly 1 inch and used for the breaking-strength measurements, which were obtained on a power-driven Scott tester with 3-inch jaws both front and back set 3 inches apart. The number of threads per inch was counted with the aid of a magnifying glass and dissecting needle on the strips prepared for the breaking-strength determinations.

Weights were determined by stamping out samples with a 2-inch square steel die and weighing them on a torsion balance calibrated to read directly in ounces per square yard. Standard thickness was measured on the same samples using the compressometer and method

developed by Schiefer (*38*).[5] Flexural work and flexural resilience were determined by the procedure outlined by Schiefer (*37*),[6] with pieces of fabric 2 by 6 inches finally cut to 2 by 4 inches.

For the determination of total sulphur 0.5-g samples were oxidized in an oxygen bomb according to a method reported by Mease (*27*). The sulphur was absorbed in a solution of ammonium carbonate and was precipitated and weighed as barium sulphate.

Nitrogen was determined on 0.1-g samples by a modified Kjeldahl method in which Gerritz's and St. John's (*19*) digestion and Winkler's (*43*) distillation procedures were used.

Three-gram samples of conditioned wool were heated to constant weight at 105° C. for the moisture determinations. During the heating process, a current of dried air was passed through the samples according to the procedure of Barritt and King (*2*).

The methylene blue absorption method of Elmquist and Hartley (*13*) was used to measure the soundness of the wool. One-tenth-gram samples of acid-washed, finely divided wool were treated with neutral, buffered, M/250 methylene blue solution and the excess of the latter titrated against naphthol yellow S. The absorption was expressed in millimols of methylene blue per 100 g of dry wool. Wool absorbs increasing quantities of methylene blue as it becomes more damaged.

Scale breakage was determined on 0.1-g samples by the Kettering modification (*24*) of the Rimington Pauly test. The samples were treated with the Pauly reagent, dissolved in sodium hydroxide, and the resulting solutions compared with a standard dye solution using a colorimeter. The intensity of the color of the solutions is assumed to be proportional to the number of scales broken. The entire determination was made in a constant-temperature laboratory at 70° F. For this test, 100 units of damage is such that a 0.1-g sample after treatment yields 25 cc of solution with the same color intensity as a 0.1-percent new acid brown S solution.

TEST RESULTS

The physical measurements gave the following average values for the serge fabric used in this investigation: Warp breaking strength, 52.3 pounds; warp thread count, 74.8 yarns per inch; warp strength index, 0.70 pound per yarn; weight, 8.4 ounces per square yard; thickness under a pressure of 1 pound per square inch, 0.0265 inch; flexural work, 19.0 thousand ergs; and flexural resilience, 63 percent.

Results of the chemical tests were: Sulphur, 3.63 percent; nitrogen, 16.26 percent; methylene blue absorption, 11.3 units; and scale breakage, 29.0 units.

Each of the above figures was obtained by averaging all the control values for the particular test involved. The use of an average control for each test condenses results and obviates the necessity of comparing each sample with its control. The percentage change from the control value due to the various treatments is shown in table 2 for all the physical tests except flexural work and resilience. The actual values for these last two measurements as distinguished

[5] Appreciation is expressed to Gladys R. White, of the Bureau of Home Economics, for making the determinations of standard thickness.

[6] Acknowledgment is made to the National Bureau of Standards for the use of their flexometer.

from percentage change are given in table 3. Table 4 reports the percentage change from the control value for the various chemical tests.

TABLE 2.—*Changes produced in some physical properties of a wool fabric as a result of various sterilizing treatments*

Treatment no.	Treatment [1]	Breaking strength [2] (warp)	Thread count [2] (warp)	Strength index [2] (warp)	Weight [2]	Thickness [3]
		Percent [4]	*Percent* [4]	*Percent* [4]	*Percent* [4]	*Percent* [4]
1	Xylene	0	+1.6	−2.7	+4.6	+20.6
2	....do	+2.1	+1.5	−.6	+6.9	+11.3
3	Autoclaving dry	−5.2	−5.1	−4.6	+2.0	+5.6
4	....do	−10.7	−2.4	−8.4	+3.3	+8.3
5	Intermittent steaming	−8.4	+1.1	−9.3	+12.4	+15.1
6	....do	−11.1	+3.5	−14.0	+12.8	+28.9
7	Autoclaving wet	−27.7	+5.1	−31.2	+17.3	−28.9
8	Formaldehyde	−10.9	+.3	−11.2	+7.6	+10.6
9	....do	−8.0	+1.3	−6.7	+7.6	+11.2
10	Mercuric chloride	−4.0	+.3	−2.4	+7.8	+14.3
11	....do	−4.0	+.3	−2.4	+10.8	+15.1

[1] For a more complete description of the treatments, see table 1.
[2] Average of 5 determinations.
[3] Average of 4 determinations.
[4] Calculated on the basis of the average control value.

TABLE 3.—*Flexural properties of a wool fabric after various sterilizing treatments*

Treatment no.	Treatment [1]	Flexural work [2] (work expended)	Flexural resilience [2] (work returned)	Treatment no.	Treatment [1]	Flexural work [2] (work expended)	Flexural resilience [2] (work returned)
		1,000 ergs	*Percent*			*1,000 ergs*	*Percent*
Control	None	19.6	63	6	Intermittent steaming.	15.1	58
1	Xylene	21.5	59				
2	....do	20.0	60	7	Autoclaving wet	23.5	50
3	Autoclaving dry	25.5	49	8	Formaldehyde	15.9	59
4	....do	29.4	53	9	....do	16.4	64
5	Intermittent steaming.	16.4	68	10	Mercuric chloride	18.9	65
				11	....do	18.4	67

[1] For a more complete description of the treatments, see table 1.
[2] 1 determination.

TABLE 4.—*Changes produced in some chemical properties of a wool fabric as a result of various sterilizing treatments*

Treatment no.	Treatment [1]	Sulphur [2]	Nitrogen [2]	Methylene blue adsorption [2]	Scale-breakage test [2]
		Percent [3]	*Percent* [3]	*Percent* [3]	*Percent* [3]
1	Xylene	+0.14	−0.18	+0.1	+4.2
2	....do	+.21	+.24	+.6	+16.1
3	Autoclaving dry	−1.10	−.31	+2.6	+15.6
4	....do	−1.53	−1.23	+4.2	+38.8
5	Intermittent steaming	−1.53	−1.78	+15.5	+135.6
6	....do	−1.69	−1.91	+19.6	+155.6
7	Autoclaving wet	−2.66	−4.38	+27.2	+165.9
8	Formaldehyde	−1.37	−.39	+15.2	(4)
9	....do	−1.51	−.43	+10.4	(4)
10	Mercuric chloride	−1.24	−1.55	−3.1	−17.7
11	....do	−2.06	−2.64	−4.6	−18.4

[1] For a more complete description of the treatments, see table 1.
[2] Average of 3 determinations.
[3] Calculated on basis of the average control value.
[4] Fabric insoluble in sodium hydroxide due to formaldehyde treatment.

APPEARANCE OF SERGE FABRICS

After sterilization, the xylene-treated serge fabrics showed a creamy color and little if any harshness. The fabrics autoclaved dry were definitely brown, harsh, and stiff. Intermittent steaming and autoclaving wet gave only a slight discoloration but a definite harshness. Formaldehyde bleached and softened the fabric. The mercuric chloride solutions caused no noticeable change in the feel but produced a slight color which became very dark and permanent when the cloth was heated.

STRENGTH INDEX

Studies published from this laboratory (*13, 22*) show that laundering oftentimes increases the breaking strength of wool fabrics. This is due to shinkage as indicated by increased thread count per inch. Therefore, a better indication of deterioration is strength index, or breaking strength per yarn, obtained by dividing the average breaking strength in pounds by the average thread count. The values thus obtained are comparable.

The 2-hour xylene treatment at 15 pounds pressure produced the smallest change in the strength index (table 2). The 1-hour treatment at 30 pounds gave a slightly larger decrease. The protective effect of the xylene is apparent when the small changes in color and strength obtained by autoclaving in xylene for 1 hour at 30 pounds pressure are compared with the brown and friable wool Walde, Barr, and Edgar (*12*) obtained after autoclaving dry, under the same conditions.

After it was found that treatment with xylene was satisfactory, strength-index measurements were made on wool sterilized by means of Stoddard solvent and tetrachlorethylene. The loss in strength index of the serge treated with Stoddard solvent at 100° C. for 12 hours was 1.1 percent and at 30 pounds for 1 hour, 3.7 percent. The value of the use of this solvent is emphasized if the above results are compared with those obtained using water under identical conditions. When the serge was heated at 100° for 12 hours in water the decrease in strength index was 29 percent and when heated at 30 pounds for 1 hour, 100 percent. With tetrachlorethylene the loss was 6.4 percent when the serge was treated at 100° for 24 hours and 4.3 percent when a pressure of 30 pounds was used for 1 hour. Thus treatment with Stoddard solvent or tetrachlorethylene gives results similar to those obtained with xylene.

Other treatments involving heat produced progressively greater changes in the order of their listing in the tables. The loss in strength index for wool autoclaved wet was more than six times that for dry wool similarly heated. This is in good agreement with results obtained by Elliott [7] who reports that "moist" and "dry" heat at 120° C. for 25 hours produced 100- and 15-percent losses, respectively, in the breaking strengths. Three intermittent steamings produced 9.3-percent loss in strength index while the four steamings increased the loss to 14 percent. Scheurer (*36*) steamed wool at 99° to 100° and likewise observed a progressive loss in strength.

[7] ELLIOTT, M. DETERIORATION IN TEXTILE FABRICS CAUSED BY HEAT AND LIGHT. 1928. (Unpublished master's thesis. Copy on file Iowa State College Library, Ames.)

Material treated with the more concentrated formaldehyde had a greater strength index than that which had been immersed in the weaker solution. Since Bell (*4*) found that the amount of formaldehyde absorbed by wool varies with the concentration of the formaldehyde solution, it would appear that absorption of formaldehyde strengthens wool. The mercuric chloride solutions produced only a very small reduction in strength index.

All treatments, except the two dry-autoclaving processes, caused an increase in thread count, indicating that some shrinkage occurred. These two gave decreases which possibly may be caused by a loosening of the weave. The formaldehyde and mercuric chloride processes gave the smallest change in thread count and the xylene treatments produced the smallest change of any means of sterilization acceptable from a bacteriological standpoint.

WEIGHT PER SQUARE YARD AND THICKNESS

Changes in the weight per square yard and thickness of the serge caused by the various sterilizing treatments were determined since variations in these properties may seriously affect the usefulness of a fabric.

Each of the treatments caused an increase in these two characteristics (table 2). Autoclaving dry or in xylene produced smaller changes than intermittent steaming or autoclaving wet. The changes resulting from the formaldehyde and mercuric chloride procedures on the whole were greater than the dry and xylene heat treatments and less than the moist. It appears that shrinkage due to the presence of moisture was the principal cause of the changes in weight per square yard and thickness.

In general, a relation exists between changes in strength index, weight per square yard, and thickness. This relationship is shown in figure 2 in which changes in strength index, weight, thickness, sulphur, nitrogen, methylene blue absorption, and scale breakage are plotted for the various heat treatments.

FLEXURAL PROPERTIES

The stiffness of a fabric is measured by the flexural work required to fold it. Flexural resilience determines the tendency of a material to unbend once it has been folded. A sterilizing treatment that produces changes in these properties obviously may be undesirable for some purposes.

Since flexural work is reported directly in ergs and flexural resilience as a percentage of flexural work, the relationship between the two is more apparent when actual values are given, as in table 3, than when percentage change from the control is listed. Table 3 shows that all of the steam-pressure treatments caused increases in flexural work, that is, they made the serge fabric stiffer. The intermittent steaming, the formaldehyde, and the mercuric chloride methods produced decreases in flexural work or softened the material.

Of the bacteriologically successful treatments, the xylene procedures gave the smallest increases in flexural work. Autoclaving dry stiffened the fabric appreciably more than autoclaving wet. The mercuric chloride processes softened the serge only slightly. The

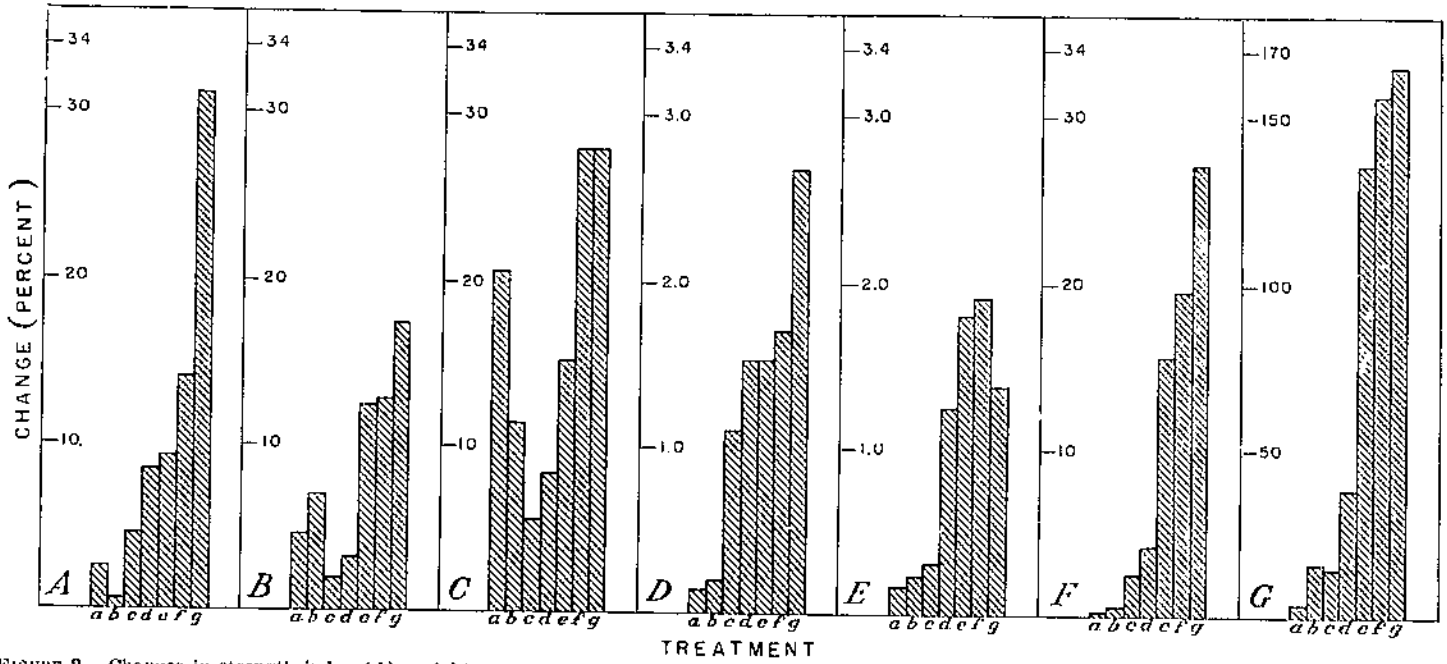


FIGURE 2.—Changes in strength index (*A*), weight per square yard (*B*), standard thickness (*C*), sulphur (*D*), nitrogen (*E*), methylene blue absorption (*F*), and scale breakage (*G*), of the serge fabric resulting from the following sterilizing treatments: *a*, Autoclaving in xylene for 1 hour at 30 pounds steam pressure; *b*, autoclaving in xylene for 2 hours at 15 pounds steam pressure; *c*, autoclaving dry for ½ hour at 15 pounds steam pressure; *d*, autoclaving dry for 1 hour at 15 pounds steam pressure; *e*, intermittent steaming for ½ hour at 100° C. on 3 successive days; *f*, intermittent steaming for ½ hour at 100° C. on 4 successive days; *g*, autoclaving wet for ½ hour at 15 pounds steam pressure.

smallest change in flexural resilience resulted from the formaldehyde and mercuric chloride treatments while autoclaving dry altered this property most.

The 10-percent formaldehyde procedure appeared to make the fabric less soft than the 2½ percent. This may be correlated with corresponding results in breaking strengths. Apparently the fabric treated with 10-percent formaldehyde is stronger than that treated with the less concentrated solution.

SULPHUR

Sulphur is present in wool protein in the form of the amino acid, cystine. The action of light, water, mineral and organic acids, alkalies, and other agencies destructive to wool reduce its sulphur content. Sulphate sulphur may be absorbed by wool from solutions used in processing or may be formed in wool by the action of various agencies on cystine sulphur. The values reported here are for cystine sulphur, as no sulphate was present. A loss in cystine sulphur is significant since it predicates wool damage.

All of the sterilizing treatments except those with xylene resulted in a loss in sulphur (table 4). In the case of the two xylene procedures the values show no decrease in the percentage of this element, the slight deviations from the control being within experimental error. Figure 2 shows that on the whole the intermittent steaming treatments caused a greater drop in sulphur content than autoclaving the wool in a dry condition, while autoclaving the fabric wet was more damaging than either. In general, the effect on sulphur of the formaldehyde and mercuric chloride procedures is comparable with that of the heat treatments.

Autoclaving wet for one-half hour at 15 pounds steam pressure caused a loss in sulphur more than twice as large as autoclaving dry under the same conditions. Similarly, Raynes (*33*) found that dry wool was not appreciably decomposed when heated in a current of dry air at 100° C. under such conditions that the moisture was retained, while moist wool seemed to be badly attacked by heating at that temperature. Woodmansey (*44*) also found that heat was not so destructive to dry wool fiber as heat in the presence of moisture.

A slightly greater loss in sulphur was found when the fabric was treated with a 10-percent solution of formaldehyde, than when a 2½-percent concentration was used.

The damaging effect of increasing the time of treatment is well illustrated in the sulphur determinations. For example, autoclaving dry for one-half hour caused a loss of 1.10 percent and the same treatment for 1 hour, a loss of 1.53 percent. Intermittent steaming on 3 successive days gave a change in sulphur of 1.53 percent and on 4 days, 1.69 percent. The decreases due to a 10- and a 30-minute mercuric chloride treatment are 1.24 percent and 2.06 percent, respectively. These various results agree with a statement of Stirm and Rouette (*39*) that the amount of hydrogen sulphide evolved when wool fibers were heated was a function of the time.

NITROGEN

Ammonia is formed during hydrolysis or oxidation of wool proteins and is lost. Since the nitrogen forming this ammonia comes

from the wool, the progress of deterioration may be followed by measuring changes in nitrogen content.

All treatments except autoclaving in xylene for 2 hours at 15 pounds pressure caused a drop in nitrogen content (table 4). Again the results of the xylene methods differ from the control value by an amount not exceeding experimental error. Sulphur and nitrogen were removed from wool by the various procedures in the ratio of approximately 1:1 (fig. 2) which is the same proportion reported by Eavenson (*12*) for the percentage loss in the two elements resulting from the first stages of attack by alkali. It should be noted that the atomic ratio of nitrogen to sulphur in wool is about 10:1.

As with sulphur, the drop in nitrogen increased with the time of autoclaving, of steaming, and of treatment with mercuric chloride. Similarly, Raynes (*33*) observed that a sample of wool lost increasing qualities of nitrogen the longer it was dried in a steam oven.

Autoclaving wet caused more change in nitrogen than autoclaving dry. However, with respect to loss of nitrogen, the two steaming processes were more damaging than pressure treatments either wet or dry.

The formaldehyde procedures had an appreciably smaller effect on the nitrogen content than did the mercuric chloride.

METHYLENE BLUE ABSORPTION

Since wool absorbs increasing quantities of methylene blue as it becomes more damaged, changes in methylene blue absorption are an indication of deterioration. Table 4 shows that the variations in methylene blue absorption resulting from the two xylene processes are again within the limits of experimental error. Except for the mercuric chloride procedures, which caused a decreased absorption, each of the remaining treatments produced a definite rise over the control value. The increases obtained for intermittent steaming agree with an observation of Fort (*16*) that the dye absorption of a sample of wool became greater after steaming.

The drop in methylene blue absorption in the case of the two mercuric chloride treatments may be due to a reaction between mercury and wool. Mills (*28*) found that when such materials as serums, enzymic preparations, and globulins were treated with mercuric chloride, a mercury proteinate was formed. The presence of mercury in wool protein might decrease the capacity of the latter for absorbing methylene blue. The mercuric chloride-treated samples, when oven-dried, turned grayish black in color.

The pronounced effect of moisture on wool during heating is shown clearly by the methylene blue values for autoclaving dry and wet. The change obtained for the former at 15 pounds steam pressure for one-half hour is 2.6 percent and for the latter under the same conditions, 27.2 percent.

The sulphur and the methylene blue-absorption tests rank the various heat treatments in the same order. For both tests, the damaging effects of the intermittent-steaming processes are more than those of autoclaving dry and less than that of autoclaving wet. As noted with sulphur and nitrogen, absorption also increases with the length of treatment.

SCALE BREAKAGE

The fibers of sound wool are covered by overlapping epidermal scales. When damage to wool occurs, some of these scales are broken off and lost. This loss decreases the resistance of the fibers to physical and chemical action and probably to attack by micro-organisms. The scale-breakage test is a measure of epidermal damage since a reaction occurs between the wool and the Pauly reagent only when the outer scales are broken.

All of the heat-sterilizing processes caused a rise in scale breakage. In general, the values for the xylene procedures are smaller than are those for autoclaving dry. The various heat treatments in the presence of moisture gave results surprisingly higher than those dry or in xylene. Autoclaving wet caused more scale breakage than intermittent steaming.

It was impossible to determine scale breakage for the formaldehyde processes since the formaldehyde-treated wool, after reacting with the Pauly reagent, would not dissolve in sodium hydroxide. According to Bell (*4*), wool immersed in a solution of formaldehyde absorbs and also combines with the formaldehyde.

The wool treated with mercuric chloride showed less scale breakage than the control samples. It is possible that the presence of mercury in the wool affects the reaction between the Pauly reagent and the cortex of the wool fibers. Mercury treatments also gave negative values for the methylene blue absorption.

Changes in methylene blue absorption and scale breakage compared with those of sulphur and nitrogen (fig. 2) emphasize the sensitivity of the former tests, especially that of scale breakage. This sensitivity has been noted by Hays, Elmquist, and Hardy (*22*) in the case of woolen blankets damaged by wear and laundering. The scale-breakage test, in addition to being highly sensitive, correlated so well with the other tests, such as sulphur, nitrogen, and methylene blue absorption, that it might serve as an indicative test method for the determination of deterioration of wool by micro-organisms.

DISCUSSION

A common soil organism, *Bacillus mesentericus*, is one of those making up the micro-organic flora of wool. It is widely distributed and has the ability to deteriorate wool. The contamination probably is caused by contact with soil and other foreign material. Since *B. mesentericus* is one of the most resistant spore formers, as well as one of the causal organisms in wool damage, it was chosen as the test organism.

A large number of sterilizing methods was tested to determine their effectiveness for killing organisms present on a woolen fabric. With each method, concentration, time, and temperature were varied. No method was discarded until it proved unsatisfactory because of the mechanical manipulations required, failure to sterilize, or undesirable physical or chemical changes produced in the wool.

Ultraviolet light, probably because of its failure to penetrate the fabric to any extent, did not kill the organisms on the wool. Such chemicals as potassium permanganate, iodine, alcohols, glycerol, tribrombetanaphthol, and tetrachlorethane under the conditions of these

experiments also were ineffective. The sodium phenylphenates did not sterilize even though the extremely small quantities diffusing into the medium were toxic to the organisms and inhibited growth at some distance from the fabric.

Formaldehyde solutions were effective sterilizing agents when used either in a 2½-percent or stronger concentration for 1 hour or in an 8-percent concentration for 30 minutes. When formalin vapors were used, it was necessary to expose the fabric for 2 hours at 70° C. None of these treatments are suitable for sterilizing wool for subsequent bacteriological studies because of bacteriostatic residues and changes produced in the fabric.

However, under certain circumstances, as for example the necessary disinfection of blankets and clothing after sickness, such treatments may be useful. The formaldehyde treatments softened and bleached the fabric. Although they produced only small changes in weight per square yard, thickness, and sulphur and nitrogen content, they caused greater changes in strength index and methylene blue absorption than any of the selected treatments except autoclaving wet and intermittent steaming. Due to an apparent reaction between formaldehyde and wool it was impossible to obtain scale-breakage results.

Treatments with mercuric salts produced sterility when used in concentrations of at least 0.4 percent at room temperature for 10 minutes. However, it was apparent that mercury was retained in the wool, as inhibition of growth upon reinoculation was very pronounced and when the wool was subsequently heated, it turned grayish black in color. Although the mercuric chloride treatments produced only small changes in the strength and flexural properties of the fabric, it appreciably increased the weight per square yard and thickness and caused relatively great losses in sulphur and nitrogen. The negative changes in methylene blue absorption and scale breakage obtained for the mercury-treated fabric suggests a reaction between wool and mercury. In general, the changes brought about by the formaldehyde and mercuric chloride treatments were smaller than the heat treatments in the presence of moisture but greater than the changes caused by heating dry and in xylene.

Four intermittent steamings at 100° C. produced sterility but made the fabric definitely harsh and weak. Except for autoclaving wet, intermittent steamings produced the greatest deterioration of the 11 selected treatments as measured by changes in strength index, weight per square yard, sulphur, methylene blue absorption, and scale breakage. The changes produced by intermittent steaming would probably influence subsequent bacteriological action.

Autoclaving the fabric in a dry condition for 30 minutes at 15 pounds steam pressure produced sterility. The physical and chemical changes were much smaller than either those produced by autoclaving the wet fabric or by intermittent steaming. This method produced only relatively small changes in strength index, nitrogen, sulphur, methylene blue absorption, and in scale breakage and it apparently did not cause shrinkage. However, it stiffened and discolored the fabric much more than any other used in this investigation.

Exposing wet wool to temperatures above the boiling point of water in an autoclave consistently produced sterility when 15 pounds

steam pressure was used for 30 minutes. The extent of the physical and chemical deterioration brought about by this treatment, however, eliminates it from consideration. It produced the greatest changes in strength index, weight per square yard, thickness, sulphur, methylene blue absorption, and in scale breakage. It appears that shrinkage due to the presence of moisture was the principal cause of the changes in weight and thickness in all of the treatments involving moisture. Losses in strength index were appreciably greater than the losses in either sulphur or nitrogen which make it probable that mechanical failure of the fabric proceeded at a greater rate than the formation of soluble degradation products.

In an effort to avoid the harmful effect of water, wool squares were immersed in a 10-percent solution of acetic acid during autoclaving. This, however, had even a greater damaging effect on the wool than water alone. Mineral oil used for the same purpose was unsuccessful. When the treatment was continued long enough, or the temperature raised high enough to produce sterility, the deterioration was obvious.

A method satisfactory from the bacteriological as well as the physical and chemical standpoints was the treatment of the wool with xylene and heat for 12 hours at 100° C., for 2 hours at 121°, or for 1 hour at 134.5°. This method resulted in sterility without leaving a bacteriostatic residue and altered the physical and chemical characteristics of the wool but slightly. Very small changes in strength index, weight, and flexural work were obtained. The variations in sulphur, nitrogen, and methylene blue absorption were within experimental error. No other treatment gave as small increases in scale breakage. It would seem that a combination of heat and immersion in an organic solvent, such as xylene, is the most effective means for sterilizing wool without changing its physical and chemical characteristics. Stoddard solvent or tetrachlorethylene can be satisfactorily substituted for xylene in the sterilization of wool.

Judging from the results obtained, it seems possible that there is a difference in the condition or the nature of the protein of the spores and that of the wool, which makes it possible to destroy the spores without damaging the wool. A differential action of hot xylene, Stoddard solvent, or tetrachlorethylene on the spores and on the wool made successful sterilization possible, whereas no such action was observed when using water, acetic acid, alcohol, or mineral oil. Such differential action may be explained by the existence of a critical region depending upon time, temperature, and the medium used.

Further investigations concerning the treatment of wool with xylene or other organic solvents with a higher boiling point than the temperature required during the sterilizing process, may show that such methods are valuable for a number of purposes. For instance, it may be possible to use xylene, Stoddard solvent, and tetrachlorethylene for removing the grease from raw wool and at the same time destroy any contamination with disease organisms, such as anthrax. Tests may show that these methods can be used to advantage in hospitals, dry-cleaning establishments, and homes for the purpose of sterilizing blankets, clothing, and other articles after contact with contagious diseases.

SUMMARY AND CONCLUSIONS

A number of sterilizing methods was tested to determine the one most satisfactory for subsequent bacteriological studies on wool fabrics using as the test organism *Bacillus mesentericus*. Physical and chemical changes in the wool fabric caused by the sterilizing treatments were measured by strength index, weight, thickness, flexural properties, sulphur and nitrogen content, methylene blue absorption, and scale breakage.

Ultraviolet light, potassium permanganate, iodine, alcohols, glycerol, tribrombetanaphthol, and tetrachlorethane did not produce sterility under the experimental conditions used. Although the sodium phenylphenates prevented growth, even when present in extremely small quantities, they did not kill the organisms.

Formaldehyde and mercuric salts gave sterility but were retained by the wool and therefore rendered the fabric unfit for subsequent bacteriological studies.

Intermittent steaming and dry and wet autoclaving produced sterility but changed the physical and chemical properties of the fabric to such an extent as to interfere with its use value.

Heating in xylene, Stoddard solvent, or tetrachlorethylene was satisfactory from the bacteriological standpoint and in general left the fabric essentially unchanged.

LITERATURE CITED

(1) AMERICAN SOCIETY FOR TESTING MATERIALS.
1936. A. S. T. M. STANDARD ON TEXTILE MATERIALS. 295 pp., illus. Philadelphia.

(2) BARRITT, J., and KING, A. T.
1926. THE SULPHUR CONTENT OF WOOL. PART 1.—INHERENT VARIATIONS ACCORDING TO THE TYPE OF WOOL. Jour. Textile Inst. 17: T386-T395, illus.

(3) BECHHOLD, H.
1909. HALBSPEZIFISCHE CHEMISCHE DESINFEKTIONSMITTEL. Ztschr. Hyg. Infektionskrank. 64: [173]-142, illus.

(4) BELL, H. S.
1927. THE ACTION OF FORMALDEHYDE ON WOOL. Jour. Soc. Dyers and Colourists 43: 76–79, illus.

(5) BENIANS, T. H. C.
1914. THE RESISTANCE OF VARIOUS BACTERIA TO THE DISINFECTING ACTION OF TOLIOL, AND THE ALLIED BODIES BENZOL AND XYLOL. Ztschr. Chemotherapie u. Verwandte Geb. 2: 28–49.

(6) BUCHANAN, R. E., and FULMER, E. I.
1930. PHYSIOLOGY AND BIOCHEMISTRY OF BACTERIA. v. 2, illus. Baltimore.

(7) BURGESS, R.
1924. STUDIES ON THE BACTERIOLOGY AND MYCOLOGY OF WOOL. Jour. Textile Inst. 15: T373–T383, illus.

(8) ———
1928. A CONTRIBUTION TO THE STUDY OF THE MICROBIOLOGY OF WOOL. Jour. Textile Inst. 19: T315–T322.

(9) CERESI, A.
1928. POTERE DISINFETTANTE DEL SUBLIMATO CORROSIVO SULLE LANE INQUINATE CON PRODOTTI TUBERCOLARI. Bol. Soc. Ital. Biol. Sper. 3: 1336–1340

(10) COULTHARD, C. E.
1936. THE RESISTANCE OF BACTERIAL SPORES TO THE BACTERICIDAL EFFECT OF MOIST HEAT AT 80° C. WITH SPECIAL REFERENCE TO THE TYNDALLISATION PROCESS OF THE BRITISH PHARMACOPOEIA, 1932. Quart. Jour. Pharm. and Pharmacol. 9: 174-187.

(11) ——— and SYKES, G.
1936. THE GERMICIDAL EFFECT OF ALCOHOL WITH SPECIAL REFERENCE TO ITS ACTION ON BACTERIAL SPORES. Pharm. Jour. 137: 79–81.

(12) EAVENSON, A.
1935. REPORT OF THE SUB-COMMITTEE ON THE CHEMISTRY OF WOOL. Amer. Dyestuff Reptr. 24: 315–316.
(13) ELMQUIST, R. E., and HARTLEY, O. P.
1935. METHYLENE BLUE ABSORPTION AS A QUANTITATIVE MEASURE OF WOOL DAMAGE. Textile Research 5: 149–156, illus.
(14) EZEKIEL, W. N., and TAUBENHAUS, J. J.
1934. COMPARING SOIL FUNGICIDES WITH SPECIAL REFERENCE TO PHYMATOTRICHUM OMNIVORUM ROOT ROT. (Abstract) Phytopathology 24: 8.
(15) ——— and TAUBENHAUS, J. J.
1935. FIELD TRIALS OF PENTACHLORETHANE, TETRACHLORETHANE, AND XYLOL AS AFFECTING PHYMATOTRICHUM ROOT ROT AND HOST PLANTS. (Abstract) Phytopathology 25: 16.
(16) FORT, M.
1916. THE EFFECT OF CERTAIN AGENCIES UPON THE COLOUR AND DYEING PROPERTIES OF WOOL AND SILK. Jour. Soc. Dyers and Colourists 32: 184–187.
(17) FULTON, D., and STANIFORD, K. J.
1918. THE STERILIZATION OF WOOLEN BLANKETS AND UNIFORMS. Jour. Amer. Med. Assoc. 71: 823–824.
(18) FURNISS, A.
1930. THE ACTION OF LIGHT ON BACTERIA. Sci. Prog. 25: 264–269.
(19) GERRITZ, H. W., and ST. JOHN, J. L.
1935. DETERMINATION OF PROTEIN NITROGEN. ACCELERATING THE KJELDAHL-GUNNING-ARNOLD DIGESTION BY ADDITION OF PHOSPHATES. Indus. and Engin. Chem., Analyt. Ed. 7: 380–383.
(20) GLOBIG.
1888. UEBER EINEN KARTOFFEL-BACILLUS MIT UNGEWÖHNLICH WIDERSTANDSFÄHIGEN SPOREN. Ztschr. Hyg. 3: [322]–332.
(21) HARRISON, F. R.
1936. CHEMICALS & WOOL. EFFECT OF VARIOUS DYEING TREATMENTS ON TENSILE STRENGTH OF WORSTED YARN. Textile World 86: 244, illus.
(22) HAYS, M. B., ELMQUIST, R. E., and HARDY, J. I.
1937. A SERVICEABILITY TEST ON BLANKETS MADE FROM FOUR BLENDS OF WOOL. U. S. Dept. Agr. Tech. Bull. 572, 24 pp., illus.
(23) HOLMES, J. F.
1936. THE ACTION OF REAGENTS UPON WOOL. Textile Colorist 58: 314–317, illus.
(24) KETTERING, J. H.
1936. A MODIFIED METHOD FOR MEASURING WOOL DAMAGE DUE TO SCALE BREAKAGE. Jour. Home Econ. 28: 255–259, illus.
(25) LATZKE, A.
1929. PENETRATION OF ULTRA-VIOLET RAYS THROUGH FABRICS. Amer. Jour. Hyg. 9: 629–645.
(26) MACDONALD, J. G.
1935. ANTHRAX IS PREVENTABLE. RECENT INVESTIGATIONS SHOW EFFECTIVENESS OF WOOL STERILIZATION AND OTHER PRECAUTIONS. Textile World 85: 2238–2239, illus.
(27) MEASE, R. T.
1934. DETERMINATION OF SULPHUR AND SULPHATE IN WOOL. Natl. Bur. Standards Jour. Research 13: 617–623. [Reprinted as Research Paper 731.]
(28) MILLS, C. A.
1925. METHOD OF STERILIZING PROTEINS OR OTHER COLLOIDAL MATERIAL. U. S. Patent no. 1,556,120. U. S. Patent Office Off. Gaz. 339: 122.
(29) MOREL, A., ROCHAIX, A., and MATHAIS, C.
1931. RECHERCHES SUR LES POUVOIRS INFERTILISANT ET ANTISEPTIQUE DE QUES HYDROCARBURES VIS-A-VIS DES CULTURES DE CERTAINS MICROBES PATHOGÈNES. Compt. Rend. Soc. Biol. [Paris] 108: 897–899.
(30) PAUL, T., and KRÖNIG, B.
1896. UEBER DAS VERHALTEN DER BAKTERIEN ZU CHEMISCHEN REAGENTIEN. Ztschr. Phys. Chem. 21: [414]–450.

(31) PRATT, F. C.
1933. WOOL AND MILDEW. Textile Recorder 51 (603) : 50–51.
(32) PRINDLE, B.
1935. THE MICROBIOLOGY OF TEXTILE FIBRES. IV. RAW WOOL. Textile Research 6 : 23–43.
(33) RAYNES, J. L.
1927. A NOTE ON THE CHEMICAL DECOMPOSITION OF WOOL AT 100° C. Jour. Textile Inst. 18 : T46–T47, illus.
(34) ROSENAU, M. J.
1922. PREVENTATIVE MEDICINE AND HYGIENE. Ed. 4, 1567 pp., illus. New York and London.
(35) SCHAFFER, J. M., and TILLEY, F. W.
1930. GERMICIDAL EFFICIENCY OF SOAPS AND OF MIXTURES OF SOAPS WITH SODIUM HYDROXIDE OR WITH PHENOLS. Jour. Agr. Research 41 : 737–747.
(36) SCHEURER, M. A.
1893. VAPORISAGE, MESURE DE L'ACTION DESTRUCTIVE QU'IL EXERCE SUR LE COTON ET SUR LA LAINE. Bull. Soc. Indus. Mulhouse 63 : 89–93.
(37) SCHIEFER, H. F.
1933. THE FLEXOMETER, AN INSTRUMENT FOR EVALUATING THE FLEXURAL PROPERTIES OF CLOTH AND SIMILAR MATERIALS. Natl. Bur. Standards Jour. Research 10 : 647–657, illus. [Reprinted as Research Paper 555.]
(38) ———
1933. THE COMPRESSOMETER, AN INSTRUMENT FOR EVALUATING THE THICKNESS, COMPRESSIBILITY, AND COMPRESSIONAL RESILIENCE OF TEXTILES AND SIMILAR MATERIALS. Natl. Bur. Standards Jour. Research 10 : 705–713, illus. [Reprinted as Research Paper 561.]
(39) STIRM, K., and ROUETTE, P. L.
1935. ÜBER DIE EINWIRKUNG VON WÄRME AUF DIE WOLLFASER. Melliand Textilber. 16 : 4–6, illus.
(40) TROTMAN, S. R., and SUTTON, R. W.
1924. THE INFLUENCE OF MICRO-ORGANISMS IN THE WEATHERING OF WOOL AND COTTON. Jour. Soc. Chem. Indus. 43 : 190T–192T, illus.
(41) ——— and SUTTON, R. W.
1925. THE DESTRUCTION OF WOOL BY EXPOSURE TO THE ATMOSPHERE. Jour. Soc. Dyers and Colourists 41 : [121]–127, illus.
(42) WALDE, E. C., BARR, M., and EDGAR, R.
1936. THE DEGRADATION OF SILK, WILD SILK AND WOOL BY STEAM. Textile Research 6 : 235–240, illus.
(43) WINKLER, L. W.
1913. BEITRAG ZUR TITRIMETRISCHEN BESTIMMUNG DES AMMONIAKS. Ztschr. Angew. Chem. 26 (Aufsatzteil) : 231–232.
(44) WOODMANSEY, A.
1918. SOME EFFECTS OF "MOIST" AND "DRY" HEAT ON WOOL. Jour. Soc. Dyers and Colourists 34 : 227–230.

ORGANIZATION OF THE UNITED STATES DEPARTMENT OF AGRICULTURE WHEN THIS PUBLICATION WAS LAST PRINTED

Secretary of Agriculture	HENRY A. WALLACE.
Under Secretary	M. L. WILSON.
Assistant Secretary	HARRY L. BROWN.
Director of Extension Work	C. W. WARBURTON.
Director of Finance	W. A. JUMP.
Director of Information	M. S. EISENHOWER.
Director of Personnel	W. W. STOCKBERGER.
Director of Research	JAMES T. JARDINE.
Solicitor	MASTIN G. WHITE.
Agricultural Adjustment Administration	H. R. TOLLEY, *Administrator.*
Bureau of Agricultural Economics	A. G. BLACK, *Chief.*
Bureau of Agricultural Engineering	S. H. MCCRORY, *Chief.*
Bureau of Animal Industry	JOHN R. MOHLER, *Chief.*
Bureau of Biological Survey	IRA N. GABRIELSON, *Chief.*
Bureau of Chemistry and Soils	HENRY G. KNIGHT, *Chief.*
Commodity Exchange Administration	J. W. T. DUVEL, *Chief.*
Bureau of Dairy Industry	O. E. REED, *Chief.*
Bureau of Entomology and Plant Quarantine	LEE A. STRONG, *Chief.*
Office of Experiment Stations	JAMES T. JARDINE, *Chief.*
Food and Drug Administration	WALTER G. CAMPBELL, *Chief.*
Forest Service	FERDINAND A. SILCOX, *Chief.*
Bureau of Home Economics	LOUISE STANLEY, *Chief.*
Library	CLARIBEL R. BARNETT, *Librarian.*
Bureau of Plant Industry	FREDERICK D. RICHEY, *Chief.*
Bureau of Public Roads	THOMAS H. MACDONALD, *Chief.*
Farm Security Administration	W. W. ALEXANDER, *Administrator.*
Soil Conservation Service	H. H. BENNETT, *Chief.*
Weather Bureau	WILLIS R. GREGG, *Chief.*

This bulletin is a joint contribution from

Bureau of Plant Industry	FREDERICK D. RICHEY, *Chief.*
Division of Soil Microbiology	C. THOM, *Principal Mycologist, in Charge.*
Bureau of Home Economics	LOUISE STANLEY, *Chief.*
Textiles and Clothing Division	RUTH O'BRIEN, *Senior Textile Chemist, in Charge.*

U. S. GOVERNMENT PRINTING OFFICE: 1937

For sale by the Superintendent of Documents, Washington, D. C. - - - - - Price 5 cents

END