



THE PERMEABILITY OF CELLOPHANE TO MICROORGANISMS

by

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Course VII

Signature of the Author.....

✓

Prof. A. L. Merrill,
Secretary of the Faculty,
Massachusetts Institute of Technology.

Dear Sir:

I submit herewith my thesis in partial fulfillment of the requirements for the degree of Bachelor of Science from the Massachusetts Institute of Technology.

Yours very truly,

176175

ACKNOWLEDGMENT

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Introduction

The protection of foods against contamination during handling and exposure for sale has greatly increased in recent years, and has led to the development of numerous types of packing materials. With the growing popularity of having foodstuffs so displayed wrapped so that the consumer can see the material he is buying, the desirability of having some sort of transparent wrapper has become of increasing importance. There are several materials which have been used for this purpose and which meet the requirements to some degree, but they all present certain problems in regard to the efficiency of protecting the product that is wrapped in them not only from the dust and dirt which might be suspended in the air, but from undue drying, and from the dangers due to the penetration by microbic organisms. One form of wrapping material that has been developed through research and which has gained great popularity during the past few years is Cellophane. The present study is an inquiry into the possible permeability of this material to certain types of microorganisms.

The word "cellophane" is the trade name for a regenerated cellulose film made by the DuPont Cellophane Company, Incorporated, and its popularity lies in the fact that it is transparent, lustrous, durable, flexible, and impervious to air. A brief history of its manufacture is given.

Spruce pulp of the finest grade is the basic ingredient. This pulp is dissolved in a caustic soda solution and carbon bisulfide is then added to produce what is known as a cellulose xanthate. To this is then added

a dilute solution of caustic soda and the resulting solution is known as viscose. After passing through a filtering and air removing process this viscose is then cast in a bath of sulphuric acid and sodium sulphate from which it emerges as a film. It is then washed and desulphonized by the use of sodium sulphide and goes through a bleaching solution of sodium hypochlorite. This film is then passed through pure glycerol which is adsorbed and tenaciously retained by the film, thus imparting softness and pliability. This completes the process and the finished film is run over drying rolls and is wound on cores into rolls of any desired weight.

This final sheet of Cellophane is approximately nine ten-thousandths of an inch in thickness, but greater thickness up to seven one-thousandths is available.

To provide a suitable wrapping material for products having a high moisture content or where it is desirable to protect them against moisture from outside sources, a special Moisture-proof Cellophane has been developed. This product is Plain Transparent Cellophane that has been processed with a microscopically thin coating of waterproofing substance which does not appreciably increase its thickness.

That this Moisture-proof Cellophane does retain or exclude moisture, as the case may be, is shown by the following table:

Materials	Diffusion of Water Vapor at 100°F.
Moisture-proof Cellophane	270 mg/m ² /hr.
Waxed brown paper	400 " "
Waxed glassine paper	530 " "
Average of fifteen waxed papers	2,000 " "

Plain Transparent Cellophane, of course, is not moisture-proof and is used in many cases as a semi-permeable membrane for dialysis of salt solutions in the separation of proteins and similar work.

Cellophane has a very high tensile strength being stronger than a good all-rag bond paper and having as high a bursting strength as the highest grade paper.

Optically, Cellophane is exceptionally transparent not only to the visible spectrum but also it passes 70% of the ultra-violet waves in the curative region of 2900 to 3100 Angstrom units. Unlike the special ultra-violet transmitting glasses which lose a large fraction of their transparency on prolonged exposure to ultra-violet rays, the transparency of Cellophane decreases but slightly after an exposure to sunlight for a year.

Cellophane finds an application in all phases of life and industry. It is used as a utility wrap on a variety of products, including baked goods, meats, frozen foods, fruits, confections, nuts, coffee, textiles, tobacco, and drugs. Each year the uses are extended into a new field so that the demand for both varieties, Moisture-proof and Plain Transparent, is constantly increasing.

Statement of the Problem

Because of the wide application of Cellophane in the food industries as a wrap for finished products, this material becomes one which concerns the public health. Therefore the object of this work is to determine the permeability of Cellophane, both Moisture-proof and Plain Transparent, to microorganisms under various conditions.

Packages wrapped in Cellophane seem to be kept in a condition free from invasion by microorganisms which might cause spoilage, but the questions that naturally arise are of great importance.

Would it not be possible for microorganisms to gain entrance to some product wrapped in either variety of Cellophane through some means as yet unaccounted for?

Can Cellophane, as such be decomposed by the action of some cellulose-attacking organism and thus give entrance to the product?

In order to answer these questions it is necessary to resort to laboratory experimentation. I, therefore, carried out the following examinations:

1. The Cellophane was examined microscopically for the presence of pores, voids, or holes of such a nature that would allow passage of microorganisms.
2. It was then put through bacteriological examinations to determine to what extent the sheets were contaminated as they were received.
3. Sterilization procedures were performed in order to determine the best method with which this Cellophane could be treated.
4. Various tests were attempted in order to determine the permeability of the material under test.

Hence, the following discussion deals with the laboratory technique, the results obtained by the various experiments, and the conclusions that were drawn from the data obtained.

Laboratory Technique

Microscopic Examination

The Plain Transparent Cellophane was examined under both high and low power with a microscope and it was found that a definite grain can be identified. This grain is due to the manner in which Cellophane is cast in the process of manufacture.

After strict search of several sheets selected at random from the stock supply no pores, holes, or any other means of passage through the sheets could be seen.

Bacteriological Examination

Several sheets of both Moisture-proof and Plain Transparent Cellophane were again selected at random, care being taken to contaminate them as little as possible. They were therefore handled with sterile tweezers and were cut into small pieces (about 1" x 2") with sterile scissors. These cut pieces were then laid on sterile agar plates and were incubated at 20°C. and 37°C. for 48 hours. At the end of this time they were examined and from the 12 plates examined four microorganisms were taken. A pair of control plates that had been exposed to air for five minutes were run for the same length of time and at the same temperature and from these plates six organisms were isolated. Upon comparison three of the organisms found on the Cellophane checked with three of those isolated from the air so it was deduced that the contamination on the Cellophane was that normally found in the air or the dust particles.

Sterilization

Because of the fact that these sheets of Cellophane were slightly

contaminated it was thought best to carry out various methods of sterilization in order to facilitate the observation of results of the later experiments.

An attempt was first made to sterilize the Cellophane by boiling it in water. Several small pieces were dropped in the water and a check was kept on the temperature. At 80°C. the Cellophane began to appear cloudy and above this temperature the cloudiness increased. This was repeated three times but each repetition was culminated by the same results. This cloudiness was probably due to the precipitation of some of the cellulose in the Cellophane but whatever the cause it was an undesirable change, so this method of sterilization was discarded.

Following this, the usual method of sterilization in an autoclave was attempted. Sheets of Cellophane were put in an autoclave and were kept at fifteen pounds pressure (about 249°F. or 121°C.) for forty-five minutes. At the end of this time they were removed and were found to have become slightly discolored and clouded. Their texture was changed so that it was brittle and upon examination with a microscope tiny cracks were visible. This procedure was repeated with the same results. As this condition of the Cellophane might render the material unsuitable for experimental work this method of sterilization was also discarded.

Sterilization by dry heat was then attempted by placing sheets of Cellophane in a hot-air oven for one-half hour at a temperature of about 180°C. At the end of this time the sheets were removed and examined and it was found that they were affected much the same as those which had been sterilized by autoclaving, except that the brittleness had been increased. Therefore, this method of sterilization was also discarded.

Attempts to sterilize the material by means of alcohol were made in the following manner: A solution of alcohol of approximately sixty percent strength was made up and sheets of Cellophane were introduced and allowed to stand for twenty-four hours. At the end of this time it was removed and washed with sterile water. As was expected, the Moisture-proof Cellophane had become milky and blistered due to the water and alcohol penetrating the waterproofing layer. The Plain Transparent Cellophane was found to be in good condition when it came out of the alcohol but upon drying it became so wrinkled that it was entirely unsuited for further work. This wrinkling was due to the uneven stresses set up in the sheets as they dried.

This same technique was carried out using the same concentration of alcohol, but a much shorter time, varying from one-half minute to two minutes. It was found that in this case the material had not been sufficiently treated as it was not sterilized. This was determined by carefully washing off the excess alcohol with sterile water and after the Cellophane had dried it was cut up and placed on poured plates of sterile nutrient agar. After 24 hours some growth was noticed. While this growth was less than that found with an untreated sheet, it was positive and this served to cause this method of sterilization to be discarded also. The same difficulties were also encountered in drying and having the sheets wrinkle.

Mercuric chloride was then used to sterilize the Cellophane, but this attempt proved fruitless because of the strength of the disinfectant which caused a change to take place in the surface of the material.

It was therefore determined to attempt to carry out these permeabil-

ity experiments by means of some microorganisms which could be easily identified and yet would not be confounded with those microorganisms normally found in air. Those organisms finally decided upon were: *Erythrobacillus prodigiosus*, *Sarcina lutea*, *Staphylococcus aureus*, *Bacillus violaceus*, *Pseudomonas aeruginosa*, *Bacterium tumefaciens*, *Bacillus cereus*, *Cellulomonas biazotea*, and *Aspergillus niger*.

The *Cellulomonas biazotea* is an organism which will break down cellulose and it was thought that its action on Cellophane might be positive. The *Aspergillus niger* is a common mold and it was made use of to determine the permeability of Cellophane to mold growths.

Experimental Procedure

Membranes of Moisture-proof Cellophane were stretched over the large end of thistle tubes and the edges were cemented down with DuPont Cellophane Cement #559.* After being allowed to dry for twenty-four hours these were examined for tightness by filling them with sterile water. Any that showed evidence of leakage were either discarded or repaired and those that proved tight were used in the test.

Twelve seventy-five cubic centimeter portions of sterile nutrient broth** were poured into 12 sterile 150 cc. beakers, to be used as the base test medium. Twelve five cubic centimeter portions of this nutrient broth were then inoculated - three with a loopful each of *Bacillus prodigiosus*, three with *Bacillus violaceus*, three with *Sarcina lutea*, and three with *Staphylococcus aureus*. These inoculated portions were carefully introduced into the small, open end of the thistle tubes, precautions being taken to avoid spilling the inoculated broth on the out-

* ** See Appendix.

side of the thistle tube where it might contaminate the broth in the beaker and therefore spoil the observation. A large cork that had been previously bored to fit the stem of a thistle tube was treated with disinfectant to remove any contamination that it might harbor. This cork was then fitted on the stem of a thistle tube and was pushed down until it reached the bulbous end. This cork was so chosen that it just fit into the beaker forming both a top for it and a stand for the thistle tube. The open end of the thistle tube was closed with sterile non-absorbent cotton and the cork was sealed to the beaker with sterile paraffine. This preparation was repeated for all twelve of the tubes. These corks all had a second hole bored in them through which a pipette could be introduced and the medium in the beaker extracted. This hole was plugged with sterile non-absorbent cotton also. The finished apparatus is shown in Fig. I of the Appendix.

These prepared tubes were incubated at twenty and thirty-seven degrees Centigrade. Observations were made hourly after eighteen hours.

After the above mentioned time it was noticed that the membrane was becoming blistered due to the penetration of the water-proofing layer by the liquid medium. Up to this time sterility of the outside medium had been maintained as was demonstrated by removing 1 cc. portions from time to time and plating them out on nutrient agar and incubating at both 20° and 37°C. for twenty-four hours. No growth on these plates showed that the inoculum had not penetrated.

After the blistering had started it was noted that the outer medium was no longer sterile. This was probably due to the fact that as the membrane blistered it caused pinholes to form by the tearing action of

the separation and the organisms found an easy exit from their hitherto tight cell.

Results

Organism	Time in hours						
	0-18	19	20	21	22	23	24
B. prodigiosus	0	0	0 blister	+	+	+	+
B. violaceus	0	blister	+	+	+	+	+
Sarcina lutea	0	0 blister	+	+	+	+	+
Staph. aureus	0	0	0 blister	+	+	+	+

Because of this blistering action this test was discarded as being performed under conditions which the material under test would never meet in practice as there is no occasion to enclose liquid media in such a limp container.

This same procedure was then repeated using the Plain Transparent instead of the Moisture-proof Cellophane.

No penetration of this Cellophane was noticed even after ninety-six hours, nor did a culture of *Aspergillus niger* inoculated in triplicate in the same manner show any penetration.

Upon completion of this experiment a series of tests were conducted which would simulate those conditions met in practice with wrapped food products and would give some definite data on how such preparations should behave.

Tubes of Cellophane were made by rolling strips (3" x 6") around a culture tube and the edges were cemented down with one of the cements* used for this purpose. This made a tube about three-fourths of an inch

* See Appendix.

in diameter and six inches in length. After being allowed to dry for twenty-four hours these tubes were then cut into two-inch lengths. One end of these short lengths was folded over and sealed with the cement, thus forming a small bag which was found to be tight by allowing water to stand in it for a short period. These were dried out by means of alcohol and ether and an air blast.

Tubes of sterile nutrient agar were then immersed in hot water for a short time so that the agar would be softened next to the wall of the test-tube and would be loosened. The agar was then caused to slide out into a sterile Petri dish where it was cut with a sterile knife into small pieces.

These blocks of agar were handled aseptically by impaling them upon a sterile nichrome inoculating needle and carefully placing them in the small bags. The blocks were then inoculated superficially with one of the test organisms and the bags were then placed on poured agar plates. This was repeated in triplicate for check purposes with each of the test organisms. These prepared plates were then incubated at 20°C. and observations were made every twenty-four hours.

The results of this experiment justified those obtained in the previous test in that the bacteria did not penetrate the Cellophane. In this case, however, the *Aspergillus niger* was noticed to be growing outside the bag. After close examination it was found that this was caused by water of condensation running out and carrying with it some of the spores of the mold. This water of condensation was due to some of the moisture evaporating from the agar and condensing on the Cellophane.

A modification of this method was then attempted. Nutrient agar was melted and poured into the small Cellophane bags until they were filled about half full. Here it was allowed to solidify and then was inoculated with each of the various cultures on the exposed surface, and by puncture with the inoculating needle in such a manner that the agar became heavily inoculated with the organism. These bags were then placed on poured plates of agar and were incubated at 20°C.

This method was very similar to that used above but it was thought that it would give a more severe test because of the greater surface of the agar that would be in contact with the Cellophane, thus giving greater possibilities of penetration.

Again trouble was encountered with the open ends allowing the water of condensation to run out and carry with it some of the organisms similar to the case cited above. Of course, this made it difficult to determine whether or not any penetration of the Cellophane had taken place. Therefore, in the subsequent runs the ends of the bags were folded over after inoculation so that any water that did condense would not run out.

These experiments showed that both Plain Transparent and Moisture-proof Cellophane were impenetrable to microorganisms.

The action of a cellulose splitting organism on Plain Transparent Cellophane was then studied. A culture of *Cellulomonas biazotea* Kellerman was obtained and was put through the same experiments as have been previously described. All inoculations were incubated at 20°C. and as this organism acts more slowly than the others the time of incubation was prolonged. Observations were made each day for eighteen days and at the end of this time no penetration was evident. It was there-

fore concluded that this organism would not penetrate the Cellophane.

Another method was used to study the action of the various organisms but it proved to be inapplicable here. Tubes of Cellophane similar to those previously described but of smaller diameter were made by rolling Cellophane strips (2" x 3") about a three-inch test tube. This made tubes one-half an inch in diameter and three inches in length. The overlapping edges were sealed with cement #559. After these had been allowed to harden for twenty-four hours they were considered ready for further manipulation. The bottoms of three-inch test tubes were cut off about one-half an inch from the bottom and the two parts so formed were cemented into the Cellophane tubes in the manner as shown in Fig. II.*

These prepared tubes were then filled with nutrient broth and were suspended in sterile nutrient broth contained in one hundred and fifty cubic centimeter wide-mouth Erlenmeyer flasks. The level of the two liquids was then made equal to obviate an unequal pressure and the liquid in the tubes was inoculated with the test organisms. These were incubated for twenty-four hours but upon first examination it was found that the liquid had caused the cement to become loosened and that there was free interchange of the liquids between the inoculated and sterile broths. Several different seals were tried but they all turned out unsuccessfully. Hence, this method was discarded.

* See Appendix.

CONCLUSIONS

My results have clearly indicated -

1. That both Plain Transparent and Moisture-proof Cellophane are impermeable to microorganisms under ordinary conditions, and as a result they are both excellent wrapping materials.

2. That the various methods of sterilization of Cellophane which we tried are unsuccessful, as they all render it unsuited for test purposes. Other methods which I believe could be applied might give favorable results but were not attempted. However, the natural bacterial flora on the Cellophane is so limited and its character is such that it led to no great difficulty in carrying out these experiments.

It was my intention to carry these experiments further with foodstuffs themselves. This might be carried out by wrapping both fresh and spoiling products in Cellophane and determining whether or not the wrappings are permeable under these conditions. It is probable that such work would prove the same thing that has been shown in this thesis but, on the other hand, some new and enlightening facts may be learned regarding this material.

The reason that this Cellophane is impenetrable to microorganisms must lie in the fact that the process of manufacture is such that no pores or interstices are formed thus giving no passageway for the bacteria or molds to utilize. The fact that it is very similar to viscose (a sodium salt of cellulose xanthate) indicates that it would not be broken down by a cellulose splitting organism since these act only on pure cellulose.

APPENDIX

YOU ARE NOW LOOKING
THROUGH
A SHEET OF
PLAIN TRANSPARENT CELLOPHANE
(0.00088 inch in thickness)

Organisms Used in Test

#1

Erythrobacillus prodigiosus (*Serratia marcescens* Bizio)
Coccobacteria: 0.5 by 0.5 to 1.0 microns, occurring singly or in pairs or in chains of 5 to 6 elements.
Motile, with peritrichous flagella. Gram-negative.
Gelatin: Liquefaction. Gray colony becoming red.
Agar Slant: White, smooth, moist, taking on a red-orange to fuchsin color.
Indol: None.
Nitrates reduced to nitrites.
Aerobic, facultative.
Optimum temperature: 25° to 30°C.
Habitat: Water, soil, milk, and foods.

#2

Sarcina lutea
Spheres: 1.0 to 1.5 microns, showing packets in all media. Gram-positive.
Gelatin: Slow liquefaction.
Agar Slant: Sulphur to chrome yellow.
Indol: Slight.
Nitrates generally reduced to nitrites.
Hydrogen sulphide formed.
Aerobic.
Optimum temperature: 25°C.

#3

Staphylococcus aureus Rosenbach

Spheres: 0.8 to 1.0 microns, single, and in pairs, or in irregular clumps. Gram-positive.

Gelatin: Liquefied.

Agar Slant: Abundant, opaque, smooth, flat, moist, yellowish to orange.

Indol: None.

Nitrates reduced to nitrites.

Slight hydrogen sulphide formation.

Pathogenic.

Aerobic.

Optimum temperature: 37°C.

Habitat: Skin and mucous membranes. The cause of boils, abscesses, etc.

#4

Chromobacterium violaceum Bergey et al. (*Bacillus violaceus* Bergonzoni).

Slender Rods: 0.8 to 1.0 by 2.0 to 5.0 microns, occurring singly or in chains.

Motile with peritrichous flagella.

Gelatin: Slight liquefaction.

Agar Slant: Deep, violet, spreading growth.

Nitrates not reduced.

Aerobic, facultative.

Optimum temperature: 25° to 30°C.

Habitat: Water.

#5

Pseudomonas Aeruginosa (Schröter) Migula
(*Bacillus pyocyaneus* Gessard)
Rods: 0.5 to 0.6 by 1.5 microns. Occurring singly, in pairs, and in short chains.
Motile, possessing one to three polar flagella. Gram-negative.
Gelatin: Rapid liquefaction.
Agar Slant: Abundant, thin, white, glistening, medium turning greenish to dark brown or black.
Indol: Not formed.
Nitrates are reduced to nitrites.
Aerobic, facultative.
Optimum temperature: 37°C.
Habitat: Open wounds, old sinuses, intestinal tract, sewage. Pathogenic to rabbits, guinea pigs, rats, and mice.

#6

Phytopomonas tumefaciens Bergey et al.
(*Bacterium tumefaciens* Smith and Townsend)
Rods: 0.6 to 1.0 by 1.2 to 1.5 microns in the infected plant and 0.7 to 0.8 by 2.5 to 3.0 microns in agar, occurring singly and in pairs.
Motile with one to three polar flagella. Rods white. Gram-negative.
Gelatin: No liquefaction.
Agar Slant: Moderate, filiform.
Indol: Formed in slight amount.
Nitrates not reduced.
Aerobic, facultative.
Optimum temperature: 25° to 28°C.
Habitat: Causes "Crown Gall" on the Paris daisy.

#7

Bacillus cereus Frankland.

Rods: 0.75 by 2.25 to 4.0 microns, occurring singly and in chains. Motile with peritrichous flagella.

Spores central, 0.8 to 1.0 by 1.125 to 1.5 microns.

Cells store glycogen as reserve material. Gram-negative.

Gelatin: moderate liquefaction.

Agar Slant: Abundant, thick, white, mealy, becoming yellowish white with pellucid dots.

Indol: Not formed.

Nitrates not reduced.

Aerobic, facultative.

Optimum temperature: 30°C.

Habitat: Soil.

#8

Cellulomonas blazotea (Kellerman) Bergey et al.

Rods: 0.5 by 0.8 micron. Motile by means of one to three peritrichous flagella. Gram-negative.

Gelatin: Liquefied.

Agar Slant: Luxuriant, yellow growth.

Indol: Not formed.

Nitrates reduced to nitrites.

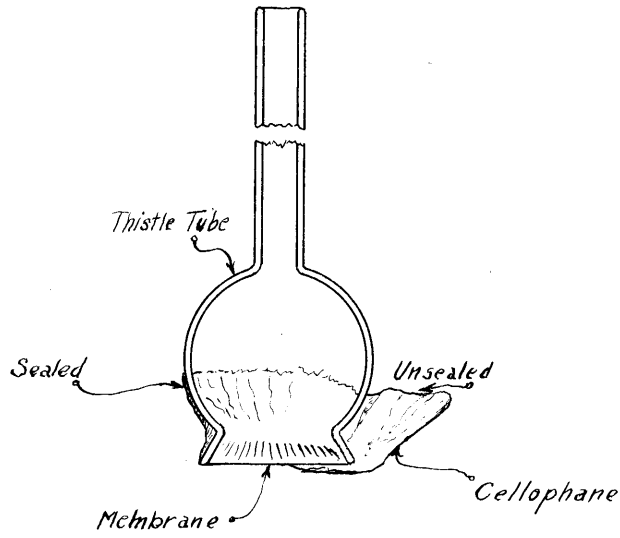
Acid in dextrose, lactose sucrose, maltose, glycerol, and starch.

Aerobic.

Optimum temperature: 20°C.

Habitat: Soil.

DIAGRAMS



Sectional Diagram of Thistle Tube Preparation.

Apparatus Set Up

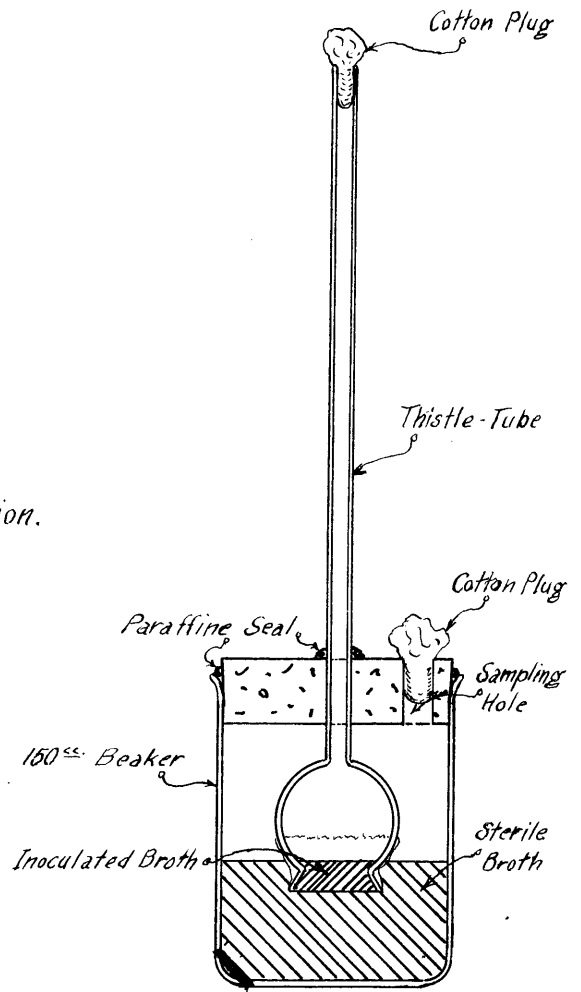


Figure I.

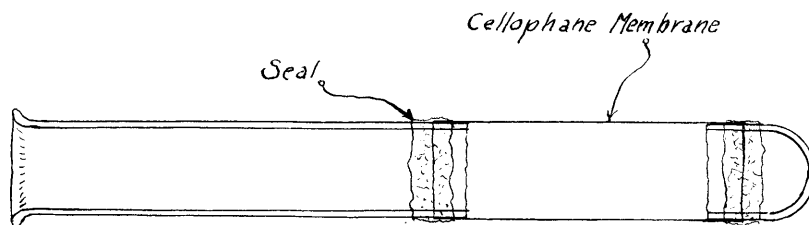


Figure II.

Media Used in Test

Nutrient Broth

Difco Nutrient Broth made up of three parts of Bacto-beef Extract and five parts of Bacto-Peptone was used. Eight grams of the above compound was mixed thoroughly with one liter of distilled water which was then placed in an autoclave and kept at a pressure of fifteen pounds for twenty minutes. At the end of this time it was removed and was tubed. The H-ion concentration of the medium did not need adjusting as that was already taken care of in the preparation of the dry medium. This was found to check with the reading given on the bottle: pH - 6.6.

Nutrient Agar

Bacto-Nutrient Agar is made up of the same ingredients as the nutrient broth with the addition of fifteen parts of Bacto-Agar. The same procedure of preparation was carried out here as in the preparation of the nutrient broth. The pH of the medium is 6.5.

Malt Extract Agar

This medium is made up of the necessary ingredients to give the same reactions as would a true malt agar. The pH when finished is 4.6.

Peptone Solution

This solution was made up from Bacto-Peptone in the required manner and the pH of the finished product was found to be 7.1.

Nitrate Agar

This agar is the same as the nutrient agar with the addition of one part of potassium nitrate. When prepared the pH was 6.7.

ADHESIVES

Some trouble was encountered with the development of an adhesive that would stick the Moisture-proof Cellophane to itself and after casting about for some such material, it was learned that the DuPont Cellophane Company, Incorporated, had developed several which would answer the purpose. Upon application to them, they were kind enough to send samples of #405, #466, #559, and Methyl-cellosolve. These were all to be used in connection with the work on Moisture-proof Cellophane. For the Plain Transparent Cellophane a sample of regular Cellophane adhesive was sent to the writer.

BIBLIOGRAPHY

1. Hyden, W. L., 1929. Manufacture and Properties of Regenerated Cellulose Films. Ind. & Eng. Chem., 21, 405.
2. Norris, J. F., 1922. Organic Chemistry. McGraw-Hill Book Co.
3. Bernthsen, A., and Sudborough, J. J., 1931. A Text-book of Organic Chemistry. D. Van Nostrand Co., Inc.
4. Bergey, D. H., 1925. A Manual of Determinative Bacteriology. The Williams & Wilkins Co.
5. Second Report of the Adhesives Research Committee. Dept. of Scientific and Industrial Research. His Majesty's Stationery Office, England.