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The Effects of Emulsions of Three Species of Acorns on Two Species of Bacteria (E. coli & S. aureus)

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THE EFFECTS OF EMULSIONS OF THREE SPECIES OF ACORNS ON TWO SPECIES OF BACTERIA

[E. coli & S. aureus]

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THE EFFECTS OF EMULSIONS OF THREE SPECIES OF ACORNS ON TWO SPECIES OF BACTERIA (E. coli & S. aureus)

by

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G.B.J.

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INTRODUCTION

Origin and Nature of the Problem

The history of medicine discloses that plant drugs have been used in all parts of the world as folk remedies, for many centuries.

Some of these drugs have been accepted for medicinal use by the medical profession while many have been disregarded because it was felt that their use was based more on superstition than on accurate and dependable information collected in regard to their therapeutic effects.

The production of penicillin, and its application in use against diseases caused by bacteria and viruses, in 1941, focused the attention of many scientists, interested in plant biology, upon the study of the effects of plant substances on the prevention and cure of diseases caused by bacteria and viruses.

Work with plant seeds, juices, stems and roots has been done by many persons in the field. Among these are Lucas and Lewis who worked with the roots of plants; Sanders and Weatherwax, who worked with the juice of plants; Little and Grubaugh who worked with the juice of plants; Cheng, Cheng, Cheng and Tong who worked with solutions of the water chestnut; Rogues who worked with leaves; and Cook who worked with juices from various parts of the pea plant.

Unpublished literature, concerned with a study of

the inhibitory effects of acorns against bacteria, undertaken by members of the Department of Natural Science, at Prairie View A. & M. College may be used as a basis for formulating a hypothesis that there is a possibility that some species of acorns may contain inhibitory factors.

Review of Important Literature on the Subject

The fact that some plants can produce factors that inhibit the growth of bacteria has been thoroughly established by studies made since 1941. These studies have revealed a number of interesting results. Some of these valuable and indispensible results which are related to the problem are brought in review and summarized below.

Ratcliff[11] reports that Sir Alexander Fleming became interested in finding a bacteria killer which would not harm the leucocytes and would not have a toxic effect in the body. In his modest laboratory at St. Mary's Hospital in London, while working on this problem, an accidental culture contamination one day set off a series of long-spaced but dramatic events which resulted in penicillin for all mankind.

While working with staphylococcus variants, a number of culture plates were set aside on the laboratory bench where they could be examined at regular intervals. The plates were necessarily exposed to air and oftentimes contamination set in. He noticed that around a large colony of a contaminating mold the staphylococcus colonies became

transparent and were obviously undergoing lysis. He decided to investigate the mold which caused the clear zone and in so doing was able to identify it as a member of the penicillium family. He then tested other varieties of mold but none except the descendents of his first colony proved so amazing.

The publication by Epstein and Williams[6] tells of the next glowing steps in this fight against disease. The next outstanding work came with the work of Sir Howard Florey of the Sir William Dunn School of Pathology, in Oxford, England. He started a study of Fleming's penicillin in 1938 and reported his findings in Lancet in 1940. The report concludes that penicillin had proved active in vivo against at least 3 of the substances which it has inhibited im the test tube experiments. He stated further that penicillin did not seem to be related to any chemical substance then being used, and that it was very successful against the anaerobic organisms of gas gangrene.

This last statement prompted much future work with the advent of World War II. Florey and N. G. Heatley came to America seeking help in this research and found it from many sources. Both men worked in this country for some time and were joined by many others who have continued the never ending search for ways of increasing the drug and purifying it.

Waksman,[18] reporting on the characteristic properties of antibiotic agents, states that some largely affect Gram positive and to a very limited extent Gram negative bacteria, while others may inhibit certain bacterial agents of each of these groups and act to a limited extent against others. He also states that antibiotic substances may vary greatly in their chemical nature, in their mode of action upon bacteria, in their toxicity to animals, and in their in vivo versus their in vitro activity. Some are destroyed by boiling, by exposure to light and by passage through various filters, whereas others are resistant to heat and to ultra violet rays.

The work done by Lucas and Lewis,[10] on the inhibitory factors found in the root systems of plants, mentioned in the folklore of numerous countries, disclosed that the inhibition of Gram positive organisms is more pronounced than that of Gram negative. They also disclose that in addition to the inhibitory effects of some plant extracts, a peculiar phenomenon of disturbed growth and very often of definite stimulation of the test organism was observed.

In their work the activity of plant extracts and their concentrates was tested by a method "similar to one described by Sherwood et. al., which was a modification of the plate assay described by British workers in connection with penicillin research." They found a similarity of anti-

bacterial action throughout a genus. However, considerable differences in the potency of the active principle were within genera and even species.

In a similar article by Sanders and Weatherwax,[13] juice of plants or particular parts of them, obtained by the Carver press method, was tested for inhibitory activity against Bacillus subtilis and E. coli using the Oxford Cup technique.

Of 150 specimen tested only 15 showed some degree of inhibitory activity against one or both test organisms. Of these no sample had been encountered which gave exceptionally high values. The most promising was the extract from the common rag-weed (Ambrosia elatior), although this was not true of the giant rag-weed (Ambrosia trifida). In many instances they found a very marked stimulation of the test organism.

The work of Little and Grubaugh,[8] with juice of leaves and stems of plants obtained by a screw press and assayed against S. aureus and E. coli, disclosed that these juices were much more active against the Gram negative organisms than against the gram positive Staphylococcus aureus.

The work done with the water chestnut, by Cheng, Cheng, Cheng and Tong[4] with the ring test method, showed positive results with S. aureus, E. coli, and Aerobacter

aerogenes. Bacillus graviolus was not affected. The active principal has been designated as "puchun" from the Chinese characters for E. lecharis tuberosa.

An article on procedure produced by the Society of Experimental Biology and Medicine,[17] suggests a thick filter paper disc saturated with the substance for easier handling. As S. aureus tends to produce a somewhat granular growth in nutrient broth, a peptone broth as the seeding medium was suggested to give a more even seeding of the test plates.

Hobby has criticized the plate method as having disadvantages arising from discrepancies in the depth and dryness of the agar. Lewis and Lucas made an attempt to control these factors by the use of a stabilized culture adapted to the seeding medium and through a more complete standardization of the preparation and treatment of the seeded plates.

They suggest also that the plates be incubated, not inverted, but placed on wooden blocks to avoid excess condensation.

Freiden's[7] report on the nature and action of antibiotics, defines an antibiotic as "a chemical substance produced by one or more less selective antimicrobial properties at relatively low concentrations. These properties may be either inhibitory or lethal".

Frieden describes the Oxford Cup method as the use

of hollow cylinders of glass, metal or porceline which are placed on agar plates, innoculated with a suitable microorganism (usually S. aureus), so as to form a liquid tight seal between the agar and the cylinder. The solution to be tested is placed in the "cup" and after incubation it is found that a circular area, in which no growth has occured, surrounds the cylinder. This method is convenient and sufficiently reliable for many purposes.

Object of Work

The objectives of this study may be stated in the following manner.

1. To provide an opportunity for the writer to get experiences in applying the scientific methods to a problem which challenged the writer's interest and stimulated her need for further study.
2. To provide opportunities to develop skills of research through techniques involving investigation, experimentation, observation and the recording of results.
3. To give the writer an opportunity to develop a more expanded and more harmonious outlook upon problems related to the field of biology.
4. To see what effect the emulsions have on the two species of bacteria (E. coli and S. aureus).
5. To provide an opportunity to develop a capacity for

meeting and solving problems independently.

A survey of the scientific literature dealing with antibiotic substances produced from plants reveals that certain plants can produce inhibitory or lethal factors which affect certain viruses and bacteria. These studies indicate the need for additional research on what has been done and research on plants that have not been previously tested. For these reasons it is apparent that there is a need for a study to determine the effects of emulsions of acorns of three types (Quercus virginiana, Quercus rubra and Quercus alba)[14] on two specific types of bacteria (Staphylococcus aureus and Escherichia coli).

The major purpose of this study was to determine whether emulsions from the hulls or meaty parts or whole acorns, of the three varieties will inhibit or stimulate the growth of S. aureus and E. coli bacteria. This involved five minor problems:

1. What variety of acorns possesses this or these factors?
2. What part of the particular variety of acorn (meaty, hulls, or whole) possesses this or these factors?
3. What concentration of the emulsion is effective?

4a. What particular bacteria is affected?

4b. Is the affected area measurable?

5. What implications does this study have upon human

welfare and diseases related to bacteria?

This study is limited to the use of three species of acorns, white oak (Quercus alba), pin oak (Quercus rubra), and live oak (Quercus virginiana). The criteria used in selection of the species were availability, adequacy, and productivity. These species of acorns are usually available for study by anyone in the Southwestern part of the United States; They are usually produced in adequate amounts each year so that continued study may be carried on if desired; Their substances are of such bulk that emulsions can easily be made. The two types of bacteria were chosen because they are available at scientific supply houses and can be readily secured at any time; they are adequate for an experiment of this type because they are representative of the Gram positive (Staphylococcus aureus) and of the Gram negative (Escherichia coli) types of bacteria. They have a rate of productivity that will lend itself to an experiment of this type.

Definitions

"Emulsions" as used here refers to minute particles of the substance dispersed throughout distilled water.

"Inhibition" is the act of checking or restraining the action of an organism.

"Stimulation" is the act of exciting or increasing

the functional activity of an organism. As used here, increased growth will result.

An "antibiotic substance" is a substance produced by certain organisms and is employed against infections caused by other organisms.[2]

Time and Place of Work

Interest began to develop in this work in September 1953, when the writer began to make observations through a review of the literature. The problem area was definitely formulated by December, at which time the writer began collecting materials which were to be used in the study.

Experimentation began early in March 1954. All laboratory work was carried on in the Natural Science Building in the biology preparatory laboratory. The experimental phase of the work was completed July 18, 1954.

MATERIALS AND METHODS

Equipment and Materials

The equipment used in this study included an autoclave, an incubator, a Waring Blendor, a refrigerator, suction filter, scales, a platinum loop, petri dishes, culture tubes, pipettes, test tube racks, test tube baskets, graduated cylinders, absorbent cotton, Bunsen burner, filter paper, discs (filter paper), forceps, jars, wax marking pencil, and a ruler marked in millimeters.

Materials used were peptone broth, nutrient agar, emulsions from the three species of acorns, the two types of bacteria and distilled water.

Procedure

Different concentrations of the emulsions were made separately from the meaty parts, the hulls, and the whole acorn of the three species by blending each with distilled water in the Waring Blendor for five minutes. The 25% solution was made by blending 1 part (1 gram) acorns to 4 parts (4 ml.) of distilled water; the 12½% solution was made by blending 1 part acorns with 8 parts distilled water; and the 6¼% solution was made by blending 1 part acorns to 16 parts distilled water. The mixtures were filtered and stored in the refrigerator in sterile jars for 12 hours before use.

The test organisms, S. aureus and E. coli were

transferred from an agar slant to a peptone broth and allowed to grow in the incubator for 24 hours at 37°C.

30 ml. of fresh nutrient agar were mixed with 1 ml. of the innoculated broth and thoroughly mixed by rolling the test tube between the palms of the hands. This mixture was then poured into a sterile petri dish which was labeled with the date, time incubation was begun, strength of the emulsion used and type of bacteria it had been innoculated with. The agar was then allowed to harden.

Filter paper discs, 21 mm. in diameter, were dipped in distilled water for the control plates seeded with bacteria (one seeded with E. coli and one seeded with S. aureus). Control plates thus prepared were designated Control N. Control A contained sterile agar and had no filter paper discs. For the remainder of the plates, discs were dipped in 25%, 12½%, and 6¼% solutions of the emulsion. Two plates were run of each strength for each type of bacteria (4 plates of 25%, 4 plates of 12½%, and 4 plates of 6¼%. Two of each group were seeded with E. coli and two of each group were seeded with S. aureus).

The filter paper discs were dipped into the filtrate and gently shaken to remove the excess filtrate before being placed on the hardened agar. Care was taken to see that the discs were not smeared over the surface of the agar but were dropped into place. Two discs were placed on each

plate. The plates were then placed in the incubator at 37°C. for 24 hours. The tops of the petri dishes were supported on ¼" corks to prevent moisture from collecting on the covers of the plates.

The plates were observed and measured with a ruler marked in mm. at 24, 48 and 72 hours. Measurements were made of the zones around the discs, and the measurements were recorded in millimeters.

EXPERIMENTS AND RESULTS

Description of the Experiment

In order to set up this experiment it was necessary to first prepare materials necessary for it. The cultures of bacteria were prepared in the following manner: The stock cultures of E. coli and S. aureus, which were purchased from a biological supply house, had to be transplanted to six tubes of agar slants. These slants were prepared as follows:

11½ grams of nutrient agar were suspended in 125 cc. of distilled water and allowed to stand a few minutes. This mixture was placed over heat and allowed to boil for two or three minutes, care being taken to see that it did not burn. The mixture was then measured so that each test tube contained 2" of media. The tubes were plugged with cotton and were placed in the autoclave where they were sterilized at 15 pounds of pressure for 15 minutes.

The autoclave was allowed to cool slowly until the pressure reached zero. The tubes were then removed and laid down so that the agar formed a long slanting surface. The tubes were allowed to cool in this position.

The bacteria were transferred from the original culture to the new sterile agar slants by means of an innoculating needle which was heated to a red glow before and after each innoculation. The mouth of the test tube

was flamed just after the cotton was removed and a loop of the culture was picked up on the innoculating needle. The mouth of the tube was flamed again and the cotton reinserted. The tube containing the fresh slant was flamed on opening and the bacteria was smeared over the surface of the slant. The tube was flamed and the cotton was reinserted. As each transplant was made the tube was labeled with the type of bacteria and the date the transplant was made.

These "planted" tubes were placed in the incubator and allowed to grow for 24 hours. They were then stored in the refrigerator.

The next step was to prepare a broth for seeding that would be suitable to mix with the agar plates.

The broth was prepared by suspending 2 grams of nutrient broth in 250 cc. of distilled water. The mixture was thoroughly heated and 15 cc. of the mixture was placed in each test tube. The test tubes were plugged with cotton and sterilized in the autoclave at 15 pounds of pressure for 15 minutes.

Bacteria was taken from the slants and implanted in the broth by the innoculating needle "loop method" described above. The tubes of broth were placed in the incubator for a 24 hour growth period.

The next step was to prepare the agar which was

to be used in the plates. This agar was prepared in the same way as the agar for the slants except that to 667 cc. of distilled water, 16 grams of agar were suspended, boiled and measured out so that each of 16 test tubes contained 30 cc. of prepared agar. These tubes were plugged and the material sterilized at 15 pounds of pressure for 15 minutes. The tubes were then allowed to cool until they could be held comfortably in the hand.

The 24 hour old broth of S. aureus was taken up into a sterile pipette and 1 cc. was extruded into each of seven of the tubes containing sterile agar. Each tube was rolled between the hands to thoroughly mix the bacteria with the agar and the mixture was then poured into a sterile petri dish.

The plates were allowed to harden while setting on a level surface.

The next step was to prepare the acorn emulsions. The acorns were prepared in the following manner. The whole acorn was cracked and weighed and placed in the Waring Blendor. For the 25% solution, to one part acorns, 4 parts of distilled water were added. These were blended for 5 minutes in the Waring Blendor. The mixture was then poured into a bottle and later was filtered and the resulting emulsion was stored in a sterile bottle in the refrigerator.

The same procedure was followed for the 12½% solu-

tion except that to one part acorns was added 8 parts of distilled water and for the 6¼% solution, to one part acorns was added 16 parts water.

In preparing the emulsions from the meaty parts of the acorns, the acorns were shelled and the shells were saved for later use. The meaty parts were weighed and for a 25% solution, 1 part acorns to 4 parts water were mixed in the Waring Blendor for 5 minutes. For a 12½% solution, 1 part acorns to 8 parts water were used and for a 6¼% solution, 1 part acorns to 16 parts water were used.

These mixtures were filtered and the filtrate was stored in the refrigerator until time for use.

The emulsions of the hulls followed the same procedure for the 25%, 12½%, and 6¼% solutions.

Solutions of only one part of one variety of acorn were made up and used on each run of plates. (i.e. Quercus alba meaty parts or Quercus rubra hulls, etc.).

DATA SHEET

Species of Acorn Used Quercus rubra (pin oaks) Part of Acorn Used Meaty parts

Ph Value of Emulsion 4.5

	First Run						Second Run					
	E. coli			S. aureus			E. coli			S. aureus		
Intervals Checked in Hours	24	48	72	24	48	72	24	48	72	24	48	72
Solutions Used (%)												
25% Plate A	N	N	N	23- 23-	23- 23-	23- 23-	N	N	N	23- 22-	23- 22-	23- 22-
25% Plate B	N	N	N	23- 23-	23- 23-	23- 23-	N	N	N	23- 22-	23- 22-	23- 22-
12½% Plate A	N	N	N	22- 22-	22- 22-	22- 22-	N	N	N	22- 22-	22- 22-	22- 22-
12½% Plate B	N	N	N	22- 22-	22- 22-	22- 22-	N	N	N	22- 22-	22- 22-	22- 22-
6¼% Plate A	N	N	N	N	N	N	N	N	N	N	N	N
6¼% Plate B	N	N	N	N	N	N	N	N	N	N	N	N
Control A	0	0	0	0	0	0	0	0	0	0	0	0
Control B	N	NN	N	N	N	N	N	N	N	N	N	N

(+) represents stimulation; (-) represents inhibition; (N) represents normal growth;
(0) represents no growth. (All measurements are in millimeters)

CHART I A

DATA SHEET

Species of Acorn Used Q. rubra (pin oaks) Part of Acorn Used Hulls

Ph Value of Emulsion 7.3

	First Run						Second Run					
	E. coli			S. aureus			E. coli			S. aureus		
Intervals Checked in Hours	24	48	72	24	48	72	24	48	72	24	48	72
Solutions Used (%)												
25% Plate A	25+	25+	25+	25+	25+	25+	25+	25+	25+	25+	25+	25+
	25+	25+	25+	25+	25+	25+	25+	25+	25+	25+	25+	25+
25% Plate B	25+	25+	25+	25+	25+	25+	25+	25+	25+	25+	25+	25+
	25+	25+	25+	25+	25+	25+	25+	25+	25+	25+	25+	25+
12½% Plate A	24+	24+	24+	24+	24+	24+	24+	24+	24+	24+	24+	24+
	23+	23+	23+	24+	24+	24+	24+	24+	24+	24+	24+	24+
12½% Plate B	24+	24+	24+	24+	24+	24+	23+	23+	23+	24+	24+	24+
	24+	24+	24+	23+	23+	23+	23+	23+	23+	24+	24+	24+
6¼% Plate A	N	N	N	N	N	N	N	N	N	N	N	N
6¼% Plate B	N	N	N	N	N	N	N	N	N	N	N	N
Control A	0	0	0	0	0	0	0	0	0	0	0	0
Control B	N	N	N	N	N	N	N	N	N	N	N	N

(+) represents stimulation; (-) represents inhibition; (N) represents normal growth;
(0) represents no growth. (All measurements are in millimeters)

CHART I B

DATA SHEET

Species of Acorn Used Q. rubra (pin oaks) Part of Acorn Used Whole acorn

Ph Value of Emulsion 4.8

	First Run						Second Run					
	E. coli			S. aureus			E. coli			S. aureus		
Intervals Checked in Hours	24	48	72	24	48	72	24	48	72	24	48	72
Solutions Used (%)												
25% Plate A	N	N	N	N	N	N	N	N	N	22+ 22+	22+ 22+	22+ 22+
25% Plate B	N	N	N	N	N	N	N	N	N	22+ 22+	22+ 22+	22+ 22+
12½% Plate A	N	N	N	N	N	N	N	N	N	N	N	N
12½% Plate B	N	N	N	N	N	N	N	N	N	N	N	N
6¼% Plate A	N	N	N	N	N	N	N	N	N	N	N	N
6¼% Plate B	N	N	N	N	N	N	N	N	N	N	N	N
Control A	0	0	0	0	0	0	0	0	0	0	0	0
Control B	N	N	N	N	N	N	N	N	N	N	N	N

(+) represents stimulation; (-) represents inhibition; (N) represents normal growth;
(0) represents no growth. (All measurements are in millimeters)

CHART I C

DATA SHEET

Species of Acorn Used Q. alba (white oak) Part of Acorn Used Meaty parts

Ph Value of Emulsion 4.82

	First Run						Second Run					
	E. coli			S. aureus			E. coli			S. aureus		
Intervals Checked in Hours	24	48	72	24	48	72	24	48	72	24	48	72
Solutions Used (%)												
25% Plate A	N	N	N	N	N	N	N	N	N	N	N	N
25% Plate B	N	N	N	N	N	N	N	N	N	N	N	N
12½% Plate A	27+ 26+	27+ 26+	27+ 26+	25+ 26+	25+ 26+	25+ 26+	27+ 26+	27+ 26+	27+ 26+	25+ 25+	25+ 25+	25+ 25+
12½% Plate B	28+ 27+	28+ 27+	28+ 27+	27+ 27+	27+ 27+	27+ 27+	27+ 27+	27+ 27+	27+ 27+	26+ 26+	26+ 26+	26+ 26+
6¼% Plate A	29+ 28+	29+ 28+	29+ 28+	27+ 28+	27+ 28+	27+ 28+	28+ 28+	28+ 28+	28+ 28+	27+ 27+	27+ 27+	27+ 27+
6¼% Plate B	29+ 29+	29+ 29+	29+ 29+	28+ 28+	28+ 28+	28+ 28+	28+ 29+	28+ 29+	28+ 29+	27+ 26+	27+ 26+	27+ 26+
Control A	0	0	0	0	0	0	0	0	0	0	0	0
Control B	N	N	N	N	N	N	N	N	N	N	N	N

(+) represents stimulation; (-) represents inhibition; (N) represents normal growth;
(0) represents no growth. (All measurements are in millimeters)

CHART II A

DATA SHEET

Species of Acorn Used Q. alba (white oak) Part of Acorn Used Hulls

Ph Value of Emulsion 6.06

	First Run						Second Run					
	E. coli			S. aureus			E. coli			S. aureus		
Intervals Checked in Hours	24	48	72	24	48	72	24	48	72	24	48	72
Solutions Used (%)												
25% Plate A	N	N	N	N	N	N	N	N	N	N	N	N
25% Plate B	N	N	N	N	N	N	N	N	N	N	N	N
12½% Plate A	25+ 25+	25+ 25+	25+ 25+	23+ 23+	23+ 23+	23+ 23+	25+ 25+	25+ 25+	25+ 25+	24+ 23+	24+ 23+	24+ 23+
12½% Plate B	25+ 25+	25+ 25+	25+ 25+	24+ 23+	24+ 23+	24+ 23+	25+ 24+	25+ 24+	25+ 24+	23+ 23+	23+ 23+	23+ 23+
6¼% Plate A	26+ 26+	26+ 26+	26+ 26+	24+ 24+	24+ 24+	24+ 24+	26+ 26+	26+ 26+	26+ 26+	24+ 24+	24+ 24+	24+ 24+
6¼% Plate B	26+ 26+	26+ 26+	26+ 26+	24+ 24+	24+ 24+	24+ 24+	26+ 26+	26+ 26+	26+ 26+	24+ 24+	24+ 24+	24+ 24+
Control A	0	0	0	0	0	0	0	0	0	0	0	0
Control B	N	N	N	N	N	N	N	N	N	N	N	N

(+) represents stimulation; (-) represents inhibition; (N) represents normal growth;
(0) represents no growth. (All measurements are in millimeters)

CHART II B

DATA SHEET

Species of Acorn Used Q. alba (white oak) Part of Acorn Used Whole acorns

Ph Value of Emulsion 5.1

	First Run						Second Run					
	E. coli			S. aureus			E. coli			S. aureus		
Intervals Checked in Hours	24	48	72	24	48	72	24	48	72	24	48	72
Solutions Used (%)												
25% Plate A	N	N	N	N	N	N	N	N	N	N	N	N
25% Plate B	N	N	N	N	N	N	N	N	N	N	N	N
12½% Plate A	N	N	N	N	N	N	N	N	N	N	N	N
12½% Plate B	N	N	N	N	N	N	N	N	N	N	N	N
6¼% Plate A	N	N	N	N	N	N	N	N	N	N	N	N
6¼% Plate B	N	N	N	N	N	N	N	N	N	N	N	N
Control A	0	0	0	0	0	0	0	0	0	0	0	0
Control B	N	N	N	N	N	N	N	N	N	N	N	N

(+) represents stimulation; (-) represents inhibition; (N) represents normal growth;
(0) represents no growth. (All measurements are in millimeters)

CHART II C

DATA SHEET

Species of Acorn Used Q. virginiana (live oak) Part of Acorn Used Meaty parts

Ph Value of Emulsion 3.85

	First Run						Second Run					
	E. coli			S. aureus			E. coli			S. aureus		
Intervals Checked in Hours	24	48	72	24	48	72	24	48	72	24	48	72
Solutions Used (%)												
25% Plate A	N	N	N	24+ 24+	24+ 24+	24+ 24+	N	N	N	24+ 24+	24+ 24+	24+ 24+
25% Plate B	N	N	N	24+ 24+	24+ 24+	24+ 24+	N	N	N	24+ 24+	24+ 24+	24+ 24+
12½% Plate A	22+ 22+	22+ 22+	22+ 22+	24+ 23+	24+ 23+	24+ 23+	22+ 22+	22+ 22+	22+ 22+	24+ 23+	24+ 23+	24+ 23+
12½% Plate B	22+ 22+	22+ 22+	22+ 22+	23+ 23+	23+ 23+	23+ 23+	22+ 22+	22+ 22+	22+ 22+	24+ 23+	24+ 23+	24+ 23+
6¼% Plate A	23+ 23+	23+ 23+	23+ 23+	22+ 22+	22+ 22+	22+ 22+	22+ 23+	22+ 23+	22+ 23+	22+ 22+	22+ 22+	22+ 22+
6¼% Plate B	23+ 23+	23+ 23+	23+ 23+	22+ 22+	22+ 22+	22+ 22+	23+ 23+	23+ 23+	23+ 23+	22+ 22+	22+ 22+	22+ 22+
Control A	0	0	0	0	0	0	0	0	0	0	0	0
Control B	N	N	N	N	N	N	N	N	N	N	N	N

(+) represents stimulation; (-) represents inhibition; (N) represents normal growth;
(0) represents no growth. (All measurements are in millimeters)

CHART III A

DATA SHEET

Species of Acorn Used Q. virginiana (live oak) Part of Acorn Used Hulls

Ph Value of Emulsion 5.5

	First Run						Second Run					
	E. coli			S. aureus			E. coli			S. aureus		
Intervals Checked in Hours	24	48	72	24	48	72	24	48	72	24	48	72
Solutions Used (%)												
25% Plate A	N	N	N	N	N	N	N	N	N	N	N	N
25% Plate B	N	N	N	N	N	N	N	N	N	N	N	N
12½% Plate A	N	N	N	N	N	N	N	N	N	N	N	N
12½% Plate B	N	N	N	N	N	N	N	N	N	N	N	N
6¼% Plate A	N	N	N	N	N	N	N	N	N	N	N	N
6¼% Plate B	N	N	N	N	N	N	N	N	N	N	N	N
Control A	0	0	0	0	0	0	0	0	0	0	0	0
Control B	N	N	N	N	N	N	N	N	N	N	N	N

(/) represents stimulation; (-) represents inhibition; (N) represents normal growth;
(0) represents no growth. (All measurements are in millimeters)

CHART III B

DATA SHEET

Species of Acorn Used Q. virginiana (live oak) Part of Acorn Used Whole acorn

Ph Value of Emulsion 5.6

	First Run						Second Run					
	E. coli			S. aureus			E. coli			S. aureus		
Intervals Checked in Hours	24	48	72	24	48	72	24	48	72	24	48	72
Solutions Used (%)												
25% Plate A	N	N	N	N	N	N	N	N	N	N	N	N
25% Plate B	N	N	N	N	N	N	N	N	N	N	N	N
12½% Plate A	N	N	N	N	N	N	N	N	N	N	N	N
12½% Plate B	N	N	N	N	N	N	N	N	N	N	N	N
6¼% Plate A	N	N	N	N	N	N	N	N	N	N	N	N
6¼% Plate B	N	N	N	N	N	N	N	N	N	N	N	N
Control A	0	0	0	0	0	0	0	0	0	0	0	0
Control B	N	N	N	N	N	N	N	N	N	N	N	N

(+) represents stimulation; (-) represents inhibition; (N) represents normal growth; (0) represents no growth. (All measurements are in millimeters)

CHART III C

Description of Results

In each series of experiments on acorns, preparation of emulsions were made from the meaty parts first, then hulls and then whole acorns. This procedure was followed for all species tested. The meaty parts were taken from the acorn and the hulls retained because the meaty parts would have dried out but the hulls could be retained with no less of value.

Six runs of the experiment were made of each species of acorns. Two on the meaty parts, two on the hulls, and two on the whole acorn. In the process of each run of each experiment, observations were made and measurements were recorded on prepared data sheets, at the end of 24, 48, and 72 hours. Observations were made to determine the stimulation (presence of increased bacterial growth) or inhibition (absence of or decreased bacterial growth) around the filter paper discs. This was done by comparing the experimental plates with the controlled plates.

Control plate A was a plate of sterile agar which showed no bacterial growth.

Control plates B were plates of agar innoculated with E. coli in one and S. aureus in the other, and on which were placed filter paper discs dipped in distilled water. These controls showed normal growth of bacteria.

Normal growth was indicated by a resemblance of

bacterial growth in the experimental plate as compared to control B where there was an equal distribution of growth of bacteria throughout the plate.

Stimulation was determined by an increase of bacterial growth around the discs of the experimental plates when compared to control plate B.

Inhibition was indicated when the area around the discs of the experimental plate was similar in appearance to control plate A in which no bacterial growth took place and the growth of bacteria outside the inhibited area was similar to the bacterial growth in control plate B.

Filter paper discs measured 21 mm. in diameter. Measurements of inhibition or stimulation were made through the combined diameter of the disc and the area around the disc.

Chart I A is a record of the experiment done on the meaty parts of Quercus rubra (pin oaks) which when tested were found to have a pH value of 4.5.

Results of these experiments (chart I A) indicate that:

1. In all plates innoculated with E. coli normal growth of bacteria occurred.
2. In plates innoculated with S. aureus, growth varied from normal to inhibition. Normal growth (bacteria growing evenly throughout the plate) occurred in

plates A and B of the 6¼% solution and inhibition occurred in plates A and B of the 12½% and 25% solutions. Measurements through the diameter of the disc and through the clear area around the discs ranged from 22 mm. to 23 mm. in the 25% solution and was constant with a measurement of 22 mm. in the 12½% solution. This seems to indicate that there was an area of inhibition of from ½ mm. to 1 mm. around the discs.

Chart I B is a record of the experiments with the emulsions of the hulls of Quercus rubra (pin oaks), which when tested showed a pH value of 7.3.

Results of these experiments (Chart I B), indicate that:

1. In plates innoculated with E. coli growth varied normal to stimulation. Normal growth occurred in plates A and B of the 6¼% solutions and stimulation occurred in plates A and B of the 12½% and 25% solutions. Measurements through the diameter of the disc and through the area of stimulation around the discs were constant with a measurement of 25 mm. in the plates with 25% solution and varied from 23 to 24 mm. in the plates with discs dipped in 12½% solutions. This seems to indicate that there was an area of stimulation from 1 mm. to 2½ mm. around the discs.

2. In plates innoculated with S. aureus, growth varied from normal to stimulation. Normal growth occurred in plates A and B of the 6¼% solution and stimulation occurred in plates A and B of the 12½% and 25% solutions. Measurements through the diameter of the discs and through the area of stimulation around the disc were constant with a measurement of 25 mm. in the plates with the 25% solution and varied from 23 to 24 mm. in the plates with discs of the 12½% solution. This seems to indicate an area of stimulation from 1 to 2½ mm. around the discs.

Chart I C is a record of the experiment with the emulsions of the whole acorns of Quercus rubra (pin oaks) which showed a pH value of 4.8.

Results of these experiments (Chart I C) indicate that:

1. In plates innoculated with E. coli, normal growth occurred in all plates.
2. In plates innoculated with S. aureus growth varied from normal to stimulation. Normal growth occurred in plates A and B of the 6¼% and 12½% solutions and stimulation in plates A and B of 1 run of the 25% solutions. Measurements through the diameter of the disc and through the area of stimulation around the disc were constant with a measurement of 22 mm. in

plates A and B of the second run of the experiment with the 25% solutions. The plates of run 1 were all normal.

A collection of data on experiments performed on the species Quercus rubra seems to point to the following tentative conclusions.

1A. Stimulation of E. coli occurred in the plates with discs dipped in 12½% and 25% solution of emulsions of the hulls of these acorns.

B. All plates of the meaty parts and whole acorns showed normal growth of E. coli.

2A. Stimulation of S. aureus occurred in the plates with discs dipped in 25% and 12½% solutions of emulsions from the hulls of these acorns.

B. Stimulation also occurred in the second run of the plates with discs dipped in 25% solution of emulsions from the whole acorn.

C. Inhibition of S. aureus occurred in the plates with discs dipped in 25% and 12½% solution of emulsions from the meaty parts of Quercus rubra acorns.

D. All other plates of S. aureus showed normal growth.

Chart II A is a record of the experiment with the emulsions of the meaty parts of Quercus alba (white oak) which had a pH value of 4.82.

Results of these experiments indicate that:

1. In plates innoculated with E. coli, growth varied from normal to stimulation. Normal growth occurred in plates with discs of the 25% solution. Stimulation occurred in plates A and B of the 12½% and 6¼% solutions. Measurements through the diameter of the discs and through the area of stimulation around the discs varied from 26 mm. to 28 mm. in the plates with 12½% solution and from 28 to 29 mm. in the plates of the 6¼% solutions. This seems to indicate that there was an area of stimulation from 2½ to 4½ mm. around the discs.
2. In the plates innoculated with S. aureus, growth varied from normal to stimulation. Normal growth occurred in plates A and B of the 25% solutions and stimulation occurred in plates A and B of the 12½% and 6¼% solutions. Measurements through the diameter of the disc and through the area of stimulation around the discs were 25 to 27 mm. in plates A and B of 12½% solution and from 26 to 28 mm. in plates A and B of the 6¼% solutions. This seems to indicate that there was an area of stimulation from 2 mm. to 4 mm. around the discs.

Chart II B is a record of the experiment with the emulsions of the hulls of Quercus alba (white oak) which

showed a pH value of 6.06. Results of these experiments indicate that:

1. Plates innoculated with E. coli, growth varied from normal to stimulation. Normal growth occurred in plates A and B of the 25% solutions and stimulation occurred in plates A and B of the 12½% and 6¼% solutions. Measurements through the diameter of the discs and through the area of stimulation around the discs varied from 24 to 25 mm. in the plates with discs of 12½% solution while a constant 26 mm. was recorded for plates with discs of the 6¼% solution. This seems to indicate that there was an area of stimulation from 1½ to 2½ mm. around the discs.
2. In the plates innoculated with S. aureus growth varied from normal to stimulation. Normal growth occurred in plates A and B of the discs dipped in the 25% solution and stimulation occurred in plates A and B of the discs dipped in 12½% and 6¼% solutions. Measurements through the diameter of the disc and through the area of stimulation around the disc varied from 23 to 24 mm. in the 12½% plates and was a constant 24 mm. in the 6¼% plates. This seems to indicate that there was an area of stimulation from 1 mm. to 1½ mm. around the discs.

Chart II C is a record of the experiment with the

emulsions of the whole acorn of Quercus alba (white oak) which showed a pH of 5.1 when tested. Results of these experiments indicate that:

1. In plates innoculated with E. coli, growth was normal in all the plates.
2. In plates innoculated with S. aureus, growth was normal in all the plates.

A collection of data on experiments performed on the species Quercus alba seems to point to the following tentative conclusions.

1A. Stimulation of E. coli occurred in the plates with discs dipped in 12½ and 6¼% solutions of emulsions of the meaty parts.

B. Stimulation of E. coli occurred in the plates with discs dipped in 12½ and 6¼% solutions of the emulsions of the hulls.

C. All other plates of E. coli showed normal growth.

2A. Stimulation of S. aureus occurred in the plates with discs dipped in 12½% and 6¼% solutions of emulsions from the meaty parts.

B. Stimulation of S. aureus occurred in the plates with discs dipped in 12½% and 6¼% solutions of emulsions of the hulls.

C. Normal growth of S. aureus resulted in all other plates.

Chart III A is a record of the experiments with emulsions of the meaty parts of Quercus virginiana (live oak) which showed a pH value of 3.85. Results of these experiments indicate that:

1. In plates innoculated with E. coli, growth varied from normal to stimulation. Normal growth occurred in plates A and B of the 25% solution and stimulation occurred in plates A and B of the 12½% and 6¼% solution. Measurements through the diameter of the discs and through the area of stimulation around the discs were constant with a measurement of 22 mm. for the plates with discs of the 12½% solution and varied from 22 to 23 mm. in the plates of 6¼% solutions. This seems to indicate that there was an area of stimulation from ½ mm. to 1 mm. around the discs.
2. In plates innoculated with S. aureus, growth showed stimulation throughout the plates. Measurements through the diameter of the discs and through the area of stimulation around the disc were constant with a measurement of 24 mm. in plates with discs of the 12½% solution. Plates with discs of the 6¼% solutions showed a constant 22 mm. This seems to indicate that there was an area of stimulation from ½ mm. to 1½ mm. around the discs.

Chart III B is a record of the experiments with the

emulsions of the hulls of Quercus virginiana (live oak) which showed pH value of 5.5. Results of these experiments indicate that:

1. In plates innoculated with E. coli, growth was normal throughout the experiment.
2. In plates innoculated with S. aureus, growth was normal throughout the experiment.

Chart III C is a record of the experiments with emulsions of the whole acorn of Quercus virginiana (live oak) which showed a pH of 5.6. Results of these experiments indicates that:

1. In plates innoculated with E. coli, growth was normal throughout the experiment.
2. In plates innoculated with S. aureus growth was normal throughout the experiment.

Collection of data on experiments performed on the species Quercus virginiana seems to point to the following tentative conclusions:

1A. Stimulation of E. coli occurred in the plates with discs dipped in the 12½% and 6¼% solutions of emulsions of the meaty parts of these acorns.

B. All parts of the hulls and whole acorns showed normal growth of E. coli.

2A. Stimulation of S. aureus occurred in the plates with

discs dipped in 25%, 12½% and 6¼% solutions of the emulsions of the meaty parts of these acorns.

B. All parts of the hulls and whole acorns showed normal growth of S. aureus.

DISCUSSION OF RESULTS

Basic Principles of Antibiotics

During the past 15 years, great strides have been made in the discovery and development of antibiotics. It has been definitely established that a number of organisms are capable of producing substances which have the power of inhibiting the growth of other organisms or even of killing them. These substances are known as antibiotics. Some antibiotics attack largely the Gram positive and to a very limited extent the Gram negative bacteria; others may inhibit certain bacteria of each of these groups and not act at all against others.[17]

Some organisms produce more than one antibiotic and some antibiotics are produced by more than one organism.

Experiments and observations have proved that factors of stimulation may be present in plant tissues along with the inhibitory factors and it is regarded possible that the inhibitory factor may lose its potency as it penetrates the agar and that it may reverse its action.[11]

It has also been proved that there are considerable differences in potency of these inhibitory and stimulative factors in their action against bacteria.[11]

In light of these basic principles that have been established concerning antibiotics and stimulating factors of plants, the study supports two main points:

1. There are substances in plants that have the power of inhibiting the multiplication of other organisms.
2. There are substances in plants along with the inhibitory factors that have the power of stimulating the growth of bacteria.

Evidence as Shown by Data

Experiments were run on 3 species of acorns to determine whether the meaty parts, hulls or whole acorns possess factors of inhibition. Experimentation and observation revealed that of the three species listed, emulsions of meaty parts of acorns of Quercus rubra (pin oaks) in 25% and 12½% solutions, had the power of inhibiting the multiplication of the Gram positive S. aureus.

Experiments and observations also revealed that of the three species tested emulsions of hulls of acorns of Quercus rubra (pin oaks) in 25% and 12½% solutions had the power to stimulate the growth of Gram negative E. coli and Gram positive S. aureus. Emulsions of the whole acorn of Quercus rubra of 25% solution had the power to stimulate Gram positive S. aureus on the second run of the experiment. In comparing the area of stimulation on this run with the area of stimulation on the runs of the hulls, the area seemed very negligible.

In comparing data on the three charts dealing with the meaty parts, hulls and whole acorns of Quercus rubra the

stimulative power attributed to the whole acorn on the 2nd run may have been due to the loss of inhibitory power of the meaty portion due to dryness of that part, or it may have been due to a faulty technique in experimentation.

Experiments and observations on Quercus alba emulsions of the meaty parts and hulls revealed that 12½% and 6¼% solutions had the power to stimulate the growth of Gram negative E. coli and Gram positive S. aureus. These data reveal that no stimulation or inhibition took place in 25% solutions of the hulls or meaty parts of Quercus alba.

Data secured from experiments and observations on Quercus virginiana emulsions of the meaty parts of acorns revealed that the 12½% and 6¼% solutions had the power to stimulate the growth of Gram negative E. coli and Gram positive S. aureus; and that the 25% solution had the power to stimulate the Gram negative S. aureus bacteria.

The solutions of these emulsions tested from a pH of 3.85 to a pH of 7.3 which was a range from acid (below 7) to slight alkalinity (above 7). No pattern of consistency could be seen in the relationship of the pH value of the solutions and the effect of the solutions on the growth of bacteria. The solution which showed inhibition had a pH value of 4.5, while these solutions that showed stimulation ranged from 3.85 to 7.3. Those that showed normal growth ranged from 4.8 to a pH value of 5.5.

Exceptional and Opposing Theories

Data revealed certain exceptions that might be explained by contrasting theories.

1. In Q. alba stimulation took place in the two lesser concentrated solutions (12½% and 6¼%) and did not occur in the more concentrated (25%) solution.
2. In Q. virginiana stimulation of E. coli took place in the two lesser concentrated solutions (12½% and 6¼%) solutions but not in the more concentrated solution. In contrast to this, stimulation of S. aureus took place in all three solutions but the higher the concentration the greater was the stimulation.

These contrasting data may be harmonized by reviewing and explaining the theories which support them.

Lucas and Lewis[10] cited Boas who calls attention to the theory that stimulative principles may be present in plant tissues together with the inhibitor and that it is regarded possible that the inhibitor as it penetrates the agar becomes dilute to such an extent that it reverts action. Another explanation may be that the power of stimulation in the less concentrated solutions is probably accounted for in that, if an inhibitory factor is present, it may become more dilute in the lesser concentrated solutions as it penetrates the agar thus freeing the stimulating factor to act.

Sanders and Weatherwax's[14] work on 150 plants seems to support the theory that if the plant has any stimulative factor at all, the higher the concentration, the greater the stimulation. Data secured from the experiment of the meaty parts of the acorn of Quercus virginiana seem to reveal that the lesser the concentration of the solutions, the greater the stimulative effect on Gram negative E. coli; but that the higher the concentration of the solution, the greater the stimulative effect upon Gram positive S. aureus. This reverse in reaction may be explained by attempting to harmonize the two contrasting theories in the following manner.

1. That a plant may contain both inhibitory and stimulative factors.
2. That the stimulating factor may act upon Gram positive and not act upon Gram negative.
3. But, that the inhibitory factor which may act on Gram negative might lose its power as the solution becomes less concentrated as the factor penetrates the agar causing a reversal of action which results in stimulation.

These observations may explain why normal stimulation occurs in S. aureus and reverse stimulation occurs in E. coli when they come in contact with emulsions of the meaty parts of Q. virginiana. (S. aureus showed more

stimulation from the stronger solutions while E. coli showed more stimulation from the 6¼% solution than from the 12½% solutions).

Comparison of Results

This work compares favorably with the works of others. Sanders and Weatherwax in testing 150 plants found 15 with an inhibitory factor which showed some degree of inhibitory activity against one of both test organisms. They state however, "that no sample has been encountered which gave exceptionally high value".[7] In this study only one part of one species (meaty parts of Q. rubra) in 25% and 12½% solutions showed inhibitory effects on only one part of one species of bacteria (S. aureus) and they did not show a large area of inhibition.

Lucas and Lewis[10] report, "very often a definite stimulation of the test organisms was observed". Sanders and Weatherwas report that "In many instances a very marked stimulation of growth of the test organism was evident". This study reveals that stimulation occurred in the 25% and 12½% solutions of emulsions of the hulls of Q. rubra against both types of bacteria and that 12½% and 6¼% solutions of meaty parts of Q. virginiana (live oaks) showed stimulation against both E. coli and S. aureus bacteria, and the 25% solution showed stimulation against only the S. aureus bacteria.

SUMMARY AND CONCLUSIONS

The major purpose of this study was to determine whether emulsions from the hulls or meaty parts or whole acorns, of the three varieties will inhibit or stimulate the growth of S. aureus and E. coli bacteria. This involves five minor problems: 1. What variety of acorns possesses this or these factors? 2. What part of the particular variety of acorns (meaty, hulls or whole) possesses the factor? 3. What concentration of the emulsion is effective? 4 a. What particular bacteria is affected? 4 b. Is the affected area measurable? 5. What implications does this study have upon human welfare and disease related to bacteria?

In light of the study these are the results applicable to the major and minor problems involved in the study.

1. Quercus rubra possessed an inhibitory factor as well as a stimulative factor. The meaty part of Q. rubra possessed the inhibitory factor in the 25% and 12½% solutions while the hulls revealed the presence of the stimulative factor in the 25% and 12½% solutions. The 25% and 12½% solutions of the meaty parts of Q. rubra affected the Gram positive S. aureus while the 25% and 12½% solution of the hulls affected both the Gram positive S. sureus and Gram negative E. coli (these results are indicated in Charts I A, B, and C).

2. Quercus alba possessed stimulative factors in the 12½% and 6¼% solutions of the emulsions of the meaty parts and in the 12½% solutions and 6¼% solutions of the emulsions of the hulls. These solutions affected the Gram positive S. aureus and the Gram negative E. coli but the area of stimulation in E. coli was about 1 mm. greater in each case than the area of stimulation in S. aureus (Charts II A, B, and C).
3. Quercus virginiana possessed a stimulative factor only in the meaty parts. The factor affected the Gram positive S. aureus in the 25%, 12½% and 6¼% solutions while the Gram negative E. coli was affected only by the 12½% and the 6¼% solutions while the Gram negative E. coli was affected only by the 12½% and the 6¼% solutions.
4. Where areas of inhibitions and stimulation were indicated, they were measured in terms of millimeters.
5. The study is far from conclusive. The results do indicate that further study is necessary to produce any significant findings that might have some effect upon human welfare and disease as related to bacteria.

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