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The incidence of Trichinella spiralis in the diaphragms of swine from the Louisville abattoirs.

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THE INCIDENCE OF TRICHINELLA SPIRALIS IN THE DIAPHRAGMS OF SWINE FROM THE LOUISVILLE ABATTOIRS

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TABLE OF CONTENTS

INTRODUCTION AND HISTORY

Trichinosis is a disease caused by the parasitic nematode Trichinella spiralis. When meat containing encysted larvae is consumed the cysts are dissolved by the action of the gastric juice in the stomach and the larvae migrate to the intestine where they mature, copulate and give birth to living young within a week of the original infection. The larvae enter the lymph spaces and are carried to the thoracic duct from which they reach the venous circulation and thence the arterial circulation by way of the heart and the pulmonary capillaries. From the arterial blood the larvae enter most of the striated muscles. After entering the muscle fibers the larvae grow rapidly, become spirally coiled, and in 4 to 6 weeks a membranous capsule begins to form around each worm. If the infested host remains alive, the cyst wall usually begins to calcify in 8 to 10 months. Eventually the entire cyst becomes calcified and the larvae die.

The presence of encysted trichinae larvae in the muscles of man was probably first noted by Tiedemann in 1822, although he failed to recognize their significance. John Hilton in 1833 was the first to suggest the parasitic nature, although the actual discovery of the worm was made in 1835 by James Paget who saw the calcified larvae in cadavers at St. Bartholomew's Hospital and found upon microscopic examination that they contained a coiled roundworm. Paget's discovery was reported by his teacher, Robert Owen, who called the parasite, Trichina spiralis.

Luckart in 1855 and Virchow in 1859 proved that larvae fed to hogs developed to adults in the host, invaded the mucosa and later encysted in the striated muscle. Zenker in 1860 was the first to recognize the physiological symptoms of trichinosis when he found both adult and larval forms in a child who had died of what was thought to be typhoid fever. It was Zenker who proved that the worm was capable of causing a serious disease and was not merely a harmless invader.

Many animals including man, swine, rats, dogs, cats, bears, and birds have been found to be infected with Trichinella spiralis. However, human infestation is almost entirely due to swine since none of the other naturally infested animals are commonly eaten by man.

Zenker's discovery of the pathogenicity of trichinae infestation coupled with Leidy's discovery of trichinae in pork focused attention upon swine as the source of human infestation. A "Medical Annotation" in the Lancet (1866) states that Professor Virchow, addressing a meeting of town councellors, butchers, doctors and laymen at Berlin in 1865 urged that microscopic examination of pork be inaugurated. He then handed the chairman of the meeting a piece of smoked sausage and a piece of meat from a trichinous pig. Urban, a veterinarian, contended that trichinae were harmless and to prove his point ate some of the infested sausage. He developed a severe case of trichinosis five days later and the furor aroused caused the butchers of Berlin to adopt microscopic examination of all pork in December, 1865. This was the first concerted attempt at control of trichinosis.

The relation of type of feed to incidence of infestation in swine was recognized by Mark (1889). In 1881 he examined 500 hogs from Chicago and found 2 per cent positive. These were a mixture of garbage and grain fed hogs. In 1888 he completed a study of 3,064 offal and garbage fed hogs from Boston and found that 12.86 per cent were infested with trichinae. In another group of 234 garbage fed hogs he found 17.95 per cent had trichinae. The procedure followed by Mark was the direct microscopic examination of 1 grain of diaphragm muscle. From a consideration of the results of his own surveys and the surveys conducted by other parasitologists Mark concluded that the type of food was the most important factor in the incidence of trichinosis in swine. He stated that a high rate of infestation was due to the feeding of garbage, offal, or any feed containing uncooked meat scraps.

De Pietra Santa (1884) in a study of trichinosis in the United States grouped the results of a number of surveys according to the type of feed received by the swine examined.

Apparently Pasture Raised Swine - 100 from Indiana, no positives (Detmers, 1883)

Southern Swine - 4146 southern swine, no positives; 241 from Louisville, Ky., 0.83 per cent positive; 5400 from New Orleans, 0.4 per cent positive (Deverson, 1881); 30 from Atlanta, Ga., no positives (Simpson, ante 1884); 180 from Nashville, Tenn., no positives (Steger ante 1884); 330 from San Antonio, Texas, 0.6 per cent positive (Meyers ante 1884).

Grain Fed Swine - At Chicago, Ill., 2 per cent positive (Atwood and Belfield, 1886); 3331 from Chicago, Ill., 2.4 per cent positive (Detmers, 1883).

Apparently Garbage Fed, Offal and Grain Fed Mixed - from Dearborn County, Ind., in 1874, 16.3 per cent positive and between 1874 - 1884, 6.5 per cent positive (Harding and Robbins ante 1884); 8773 from Boston, Mass., 4.0 per cent positive (Billings, 1879 - 81); 529 from St. Louis, Mo., 3.4 per cent positive (Deverson, 1881).

Calvin (1890) reported that approximately 8 - 10 per cent of the swine in Iowa fed on slaughterhouse offal were infested with trichinae while the grain fed hogs were practically free of trichinosis.

During the 15 years from 1891 - 1906 the United States required that all export pork be examined microscopically for trichinosis. Of 8,257,928 diaphragms examined between 1898 and 1906 approximately 1.5 per cent were found to be infested with living trichinae larvae. An additional 1 per cent were found to contain dead trichinae or bodies that resembled trichinae.

Schwartz has reported the examination of 25,000 diaphragms during the years 1933 - 38. The average incidence of infestation in 13,000 farm raised hogs was 0.95 per cent and in 10,300 garbage fed hogs was 5.7 per cent. The digestion method of examination was employed.

Hobmaier and Geiger (1938) in a survey of trichinosis of swine at San Francisco found 1.4 per cent of 92 garbage fed hogs contained trichinosis but found no trichinae in 108 hogs with unknown type of feed. The digestion method was used.

Barrett and Sears (1938) reported 194 Michigan swine examined by the digestion method with no positives.

Cameron (1938 - 40) has published three reports of the incidence of trichinosis in Canada as determined by the digestion technique. In the first report 729 hogs had been examined and 2.06 per cent were found positive. In the next year he found an incidence of infection of 0.75 per cent in 2,000 hogs and in the third group studied only 0.2 per cent of the 995 hogs were infested.

Hood and Olsen (1939) examined 500 grain fed hogs by the digestion method and found 0.4 per cent positive.

Kerr and Jacobs (1940) reported an incidence of 0.5 per cent in 566 grain fed hogs, 6.0 per cent in 502 garbage fed hogs and 1.0 per cent in 1532 hogs with mixed and unknown feed.

McNaught and Zapata (1941) examined 495 garbage fed hogs at San Francisco by both the microscopic and digestion methods and found 4.04 per cent positive.

Although there have been numerous reports on the incidence of trichinosis in swine in the United States, a study of all available data indicates that the incidence varies greatly with the locality and the predominant type of feeding. For this reason it was considered important to obtain data on the incidence of trichinosis in swine slaughtered in Jefferson County, Kentucky.

SURVEY OF METHODS

There are three methods which have been used extensively for the detection of trichinae infestation, the direct microscopic method, the digestion-Baermann method and the intradermal test.

Direct Microscopic Technique

The direct microscopic technique consists of placing small pieces of the muscle to be examined between two glass plates, pressing the plates together so that a uniform transparency of the muscle fibers is obtained and examining under the microscope with the low power objective. The trichinae press most commonly used at present is the one described by Nolan Bozicevich (1938). It consists of two glass plates approximately 8 3/4 inches long by 2 inches wide and ¼ inch thick which are pressed between two metal frames by four bolts and nuts. This press differs from the one described by Hall and Collins (1937) and previously used by the majority of workers in this field only in that the earlier type was fitted with two bolts and nuts instead of four. The four bolt arrangement is said to give a more uniform transparency of the fibers.

There has been considerable variation in the amount of muscle examined by different authors. During the years 1891 - 1906 pork intended for export to certain European countries was examined for trichinae by Federal meat in-

spectors. The recognized procedure at that time was to examine three small samples, each about the size of an oat grain. The laboratory methods of the U. S. Army require that only one sample, the size of a pea, be examined. Hall and Collins and other parasitologists of the U. S. Public Health Service have used one gram portions in all of their surveys.

Digestion-Baermann Technique

Numerous modifications of the digestion-Baermann technique have been used. However, most methods are very similar to the one described by Hall and Collins (1937). The diaphragm is weighed, ground through an ordinary meat grinder and digested at 37°C in an artificial digestive juice composed of 5 grams of pepsin and 7 cc. of concentrated hydrochloric acid per liter of water. The mixture is stirred every half hour with a glass rod for the first two or three hours and left in the incubator for 18 hours or longer. At the end of the digestion period most of the supernatant fluid is siphoned off and the remaining fluid poured into a Baermann apparatus (a large funnel with an 80 mesh screen fitted into the top and closed at the base with a piece of rubber tubing and a Hoffman clamp). After the fluid is allowed to stand one hour or longer, about 200 cc. are drawn off and examined under the low power objective of the microscope for trichinae larvae. These authors state that 3 liters of arti-

ficial gastric juice are required for proper digestion of samples weighing 50 grams or more.

Cameron (1938) used only 625 cc. of 0.2 per cent pepsin solution maintained at a pH of 1.0 - 2.0 by the addition of HCl for the digestion of a 50 gram sample. It is very probable that Cameron used mechanical agitation to obtain proper digestion with this reduced amount of fluid though he made no statement to that effect in his paper. Cameron also found that 0.05 gm. of papain dissolved in 150 cc. of normal saline could be substituted for the artificial gastric juice.

As with the direct method, the greatest amount of variation in the digestion technique has been in the amount of material examined. Hall and Collins (1937) and many others making post-mortem examinations of human diaphragms digested the entire diaphragm. The average weight was 98 grams. Queen (1931), McNaught and Anderson (1936), and others have used 50 grams of diaphragm in their surveys. Cameron in his studies of hog diaphragms digested only 10 gram portions. Hood and Olsen (1939) in a survey of trichinosis of swine in the Chicago area devised a system of mass digestion. They secured 20 gram samples, retained 10 grams and combined 10 grams with 19 other samples. The whole was then digested and examined. If positive the twenty were then examined individually.

The Skin Test

At the present time the value of the intradermal test for the detection of trichinae infestations in swine is a controversial question. Schwartz (1941) stated that the reactions produced by the intracutaneous injection of T. spiralis antigens are not sufficiently reliable and clear cut to be applicable to meat inspection. He based this statement on the results of over 11,000 tests conducted by parasitologists of the Bureau of Animal Industry. Lichterman and Gleeman (1939) reported the results of skin tests of 211 garbage fed hogs. These tests were checked by post-mortem examination of the diaphragms and it was found that 206 out of the 211 or 97 per cent had been diagnosed correctly. In additional tests made by these authors on 126 hogs, only two were diagnosed incorrectly.

It may be said that a rapid, specific skin test may soon be available but that much more study is needed before this test can be included in the routine inspection of swine to be slaughtered.

ADAPTATION OF METHODS

The 1000 diaphragms studied in this survey were examined by both the direct microscopic technique and the digestion-Baermann technique.

Slight modifications were made in both methods. In the direct microscopic examination the facia was first removed and 1/2 gram of muscle from various parts of the diaphragm was cut into small thin strips. These strips were then placed in the "trichinae press" and examined under the low power objective of the microscope. The press used in this study consisted of two pieces of plate glass 2" x 10" x 1/4" pressed together by a screw clamp at either end (Fig. I). It was found that the transparency of muscle fibers obtained with this press was comparable to that obtained with the press described by Hall and Collins and Nolan and Bozicevich.

In the digestion-Baermann technique the tendenous and fatty portions of the diaphragm was put through an ordinary meat chopper. The sample was then digested in 3 liters of artificial gastric juice (5 grams pepsin and 7 cc. concentrated HCl per liter of water). The digestions were set up in a 37°C incubator fitted with electric agitators (Fig. II) so that the material was stirred constantly for 24 hours. At the end of this time the fluid was poured into a modified Baermann funnel (Fig. III). The Baermann apparatus consisted of a large funnel closed at the bottom

by means of a piece of rubber tubing and a screw clamp. The top of the funnel was fitted with a double layer of gauze instead of the usual 80 mesh screen to retain any pieces of tendon and undigested fibers. The fluid was allowed to settle in the funnel for two hours and then portions were drawn off into Petri dishes and examined for larvae.

After 107 diaphragms had been examined it was decided to adopt the mass digestion procedure of Hood and Olsen. Tendon and fat were removed from the diaphragms and 10 gram portions from each of 10 or 20 diaphragms were digested together and the remainder of the diaphragms were retained so that they might be run individually if larvae were found in the mass digestion.

In the course of this investigation it was found that complete digestion of the muscle could be obtained with less than 3 liters of digestive fluid for 50 grams of sample. In the latter part of this work 1000 cc. of artificial gastric juice was used to digest 50 grams of diaphragm. In all probability the majority of workers in this field have overlooked the possibility of reducing the amount of digestive fluid because they relied on occasional manual agitation with a stirring rod instead of mechanical agitation.

RESULTS AND DISCUSSION

Of the 1000 diaphragms examined only two positives were found by the digestion method and one by the direct microscopic method. The one found positive by the direct examination was also positive by digestion. Thus the incidence of infestation in the group studied is 0.2 per cent. (Table I)

This discrepancy between the results of the direct and the digestion methods is to be expected. Hall and Collins found that either method alone failed to detect trichinae in a certain number of cases. This view was supported by Nolan and Bozicevich who stated that the microscopic method failed in a number of cases of light infestation. They concluded that though the digestion method was valueless in detecting dead trichinae it was reliable in detecting even very light infestations in which living worms were present.

The diaphragms were collected from four abattoirs in Louisville, Kentucky over a period of nine months and therefore should represent a true cross-section of the incidence of trichinae-infested pork reaching the Louisville markets.

The incidence of infestation of swine with T. spiralis in Jefferson County is considerably lower than the average incidence of 1.5 per cent for the United States as estimated by Schwartz. The correlation of trichinosis in swine

with the predominant type of feeding in the various geographical sections of the United States, however, indicates that the 0.2 per cent infestation is very little lower than should be expected. Hall (1937) stated that the incidence of trichinosis in swine is approximately as follows: Pasture-raised swine mostly in the Middle West are free or practically free from trichinae. The so-called grain fed swine in the Middle West are in reality a mixture of some pasture raised and some garbage fed swine and the mixture has an average infestation of about 1.5 per cent. Southern swine have an average infestation of less than 1 per cent. Garbage fed hogs which are most numerous along the southern part of the Pacific coast and the northern part of the Atlantic coast have an average incidence of about 5 per cent. The last group, and the one which has practically disappeared, is the offal fed hog which had the highest incidence of infestation, about 18 per cent.

The swine examined in the present study would be classed by Hall and others as grain fed for although we were unable to determine the exact feeding, these swine were raised in a section of the country in which grain feeding predominates and were purchased for slaughter in the belief that they had been grain fed.

In Table II the results of this survey are compared with the results of several recent surveys of grain fed swine. It is evident that with such a small number of

positives a difference of several tenths of a per cent is not significant.

The data summarized in Table III reveals that a number of workers have reported examining a series of hog diaphragms without finding any infested with T. spiralis, thus further substantiating the belief that the reported incidence of 0.2 per cent for Jefferson County is not unusually low.

SUMMARY

In the examination of 1000 diaphragms collected from swine slaughtered in Louisville abattoirs, 2 were found infested with trichinae by the digestion-Baermann method and one of these was also found infested by the direct microscopic method, an incidence of 0.2 per cent.

Although the exact type of feed is unknown these swine may be classified as grain-fed in conformity with the method used by Hall and Schwartz of classifying swine according to the type of feeding most prevalent in the section of the country in which the swine were raised.

The incidence reported here is lower than the average of 1.5 per cent for the United States as estimated by Schwartz but many other investigators have reported similarly low degrees of infestation.

TABLE I

Incidence of *Trichinella spiralis* in the Diaphragms of Swine from the Louisville Abattoirs

Source	No. Examined	No. found infested by Direct Method	No. found infested by Digestion Method	Per cent found infested by Direct Method	Per cent found infested by Digestion Method
Abattoir #1	505	1	2	0.2%	0.4%
Abattoir #2	241	0	0	0	0
Abattoir #3	139	0	0	0	0
Abattoir #4	115	0	0	0	0
Total	1000	1	2	0.1%	0.2%

TABLE II

Incidence of Trichinosis in Grain-Fed Swine

as Indicated by Recent Surveys

Author	Year	Method of Examination	No. Examined	No. Infested	Per Cent Infested	Location
Kerr and Jacobs	1940	digestion	566	3	0.5	California
Schwartz	1936	digestion	4740	53	1.0	
Schwartz	1940	digestion	4461	33	0.76	
Hood and Olsen	1939	digestion	500	2	0.4	Chicago, Ill.
Adams	1942	microscopic and digestion	1000	2	0.2	Louisville, Ky.

TABLE III

Incidence of Trichinosis in Swine

Author	Year	Type of Feed	Method of Examination	No. Examined	No. Infested	Per Cent Infested	Location of Survey
Mark	1881	grain and garbage	microscopic	500	10	2.0	Chicago, Illinois
Mark	1888	garbage offal	microscopic	3064	391	12.86	Boston, Massachusetts
Mark	1888	garbage	microscopic	234	42	17.95	Massachusetts
Calvin	1890	offal				8 – 10%	Iowa
Calvin	1890	grain				practical-ly none	Iowa
Federal Inspection	1898 1906		microscopic	8,257,928		1.5% alive 1.0% dead	Entire U. S.
Billings	1880	grain and cooked meat	microscopic	28	0	0	Massachusetts
Billings	1879 – 1881	garbage offal and grain		8773	347	4.0	Boston, Massachusetts
Deverson	1881			4146	0	0	Southern U. S.
Deverson	1881			241		0.83	Louisville, Kentucky
Deverson	1881			5400	22	0.4	New Orleans, Louisiana

TABLE III (Con't.)

Incidence of Trichinosis in Swine

Author	Year	Type of Feed	Method of Examination	No. Examined	No. Infested	Per Cent Infested	Location of Survey
Deverson	1881			529		3.4	St. Louis, Missouri
Detmers	1883	pasture raised		100	0	0	Indiana
Detmers	1883	grain				2.0	Chicago, Illinois
Simpson	ante 1884			30	0	0	Atlanta, Georgia
Steger	ante 1884			180	0	0	Nashville, Tennessee
Meyer	ante 1884			330		0.6	San Antonio, Texas
Atwood and Belfield	1886	grain				2.0	Chicago, Illinois
Harding and Robbins	1874	garbage offal and grain				16.3	Dearborn Co., Indiana
Harding and Robbins	1874 – 1884	garbage offal and grain				6.5	Dearborn Co., Indiana

TABLE III (con't.)

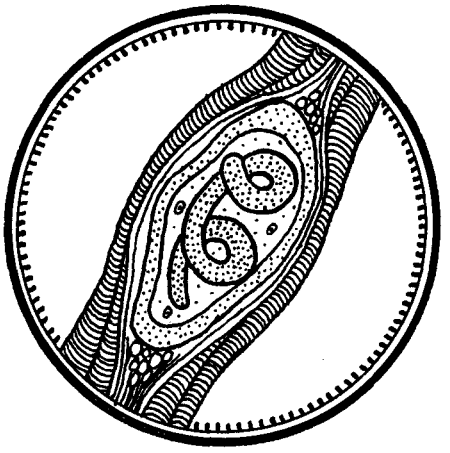
Incidence of Trichinosis in Swine

Author	Year	Type of Feed	Method of Examination	No. Examined	No. Infested	Per Cent Infested	Location of Survey
McNaught and Zapata	1941	garbage	microscopic and digestion	495	20	4.04	San Francisco, California
Hobmaier and Geiger	1938	garbage	digestion	92	13	1.4	San Francisco, California
Hobmaier and Geiger	1938	unknown	digestion	108	0	0	San Francisco, California
Kerr and Jacobs	1940	grain	digestion	566	3	0.5	California
Kerr and Jacobs	1940	garbage	digestion	502	30	6.0	California
Kerr and Jacobs	1940	other than garbage or grain	digestion	1532	14	1.0	California
Schwartz	1936	garbage	digestion	2341	130	5	
Schwartz	1936	grain	digestion	4740	53	1.0	
Schwartz	1938	garbage	digestion	2847	286	10.0	

TABLE III (con't.)

Incidence of Trichinosis in Swine

Author	Year	Type of Feed	Method of Examination	No. Examined	No. Infested	Per Cent Infested	Location of Survey
Schwartz	1938	cooked garbage	digestion	1860	11	0.59	
Schwartz	1940	grain	digestion	4461	33	0.75	
Schwartz	1940	garbage	digestion	5312	136	2.6	
Hood and Olsen	1939	grain	digestion	500	2	0.4	Chicago, Illinois
Barrett and Sears	1938			194	0	0	Michigan
Cameron	1938		digestion	729	15	2.06	Canada
Cameron	1939	cooked garbage	digestion	2000	15	0.75	Canada
Cameron	1940		digestion	995	2	0.2	Canada



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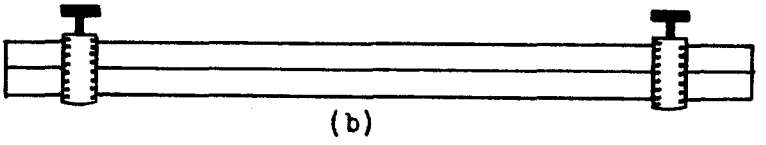

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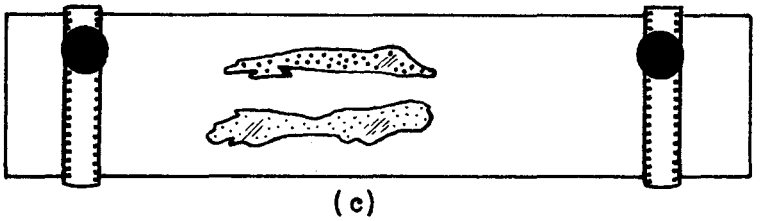

(c)

FIGURE I

(a) Larvae of Trichinella spiralis
(b) Trichinae Press (side view)
(c) Trichinae Press (top view)

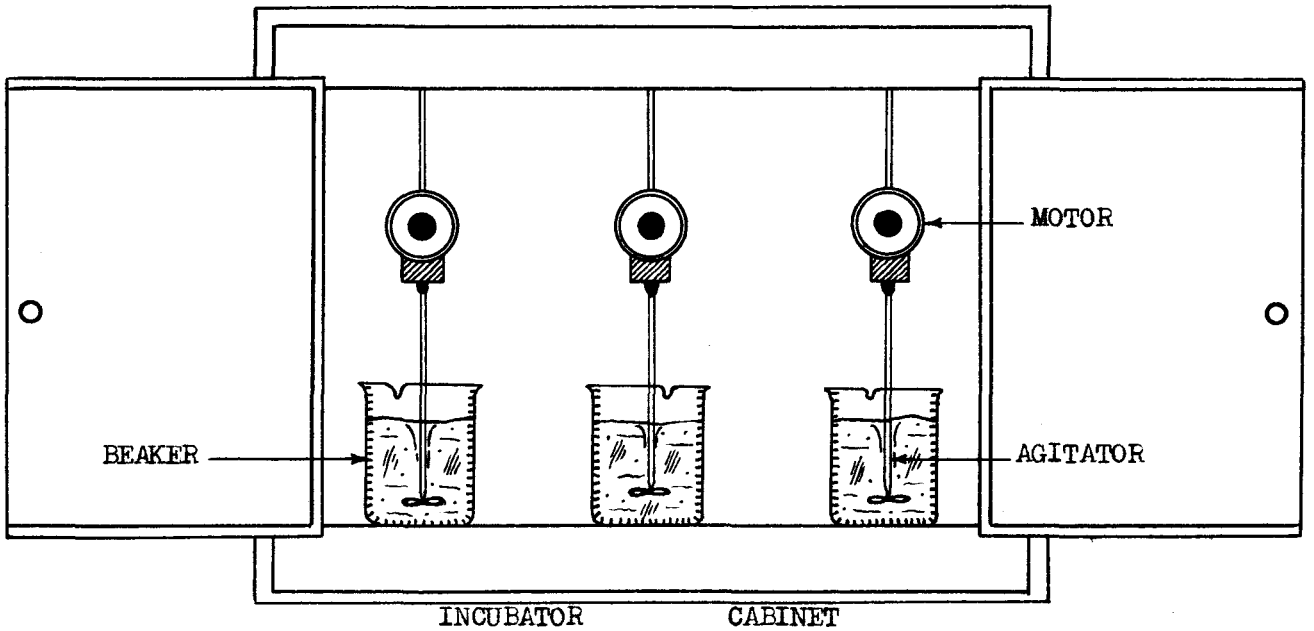

FIGURE II

DIGESTION APPARATUS

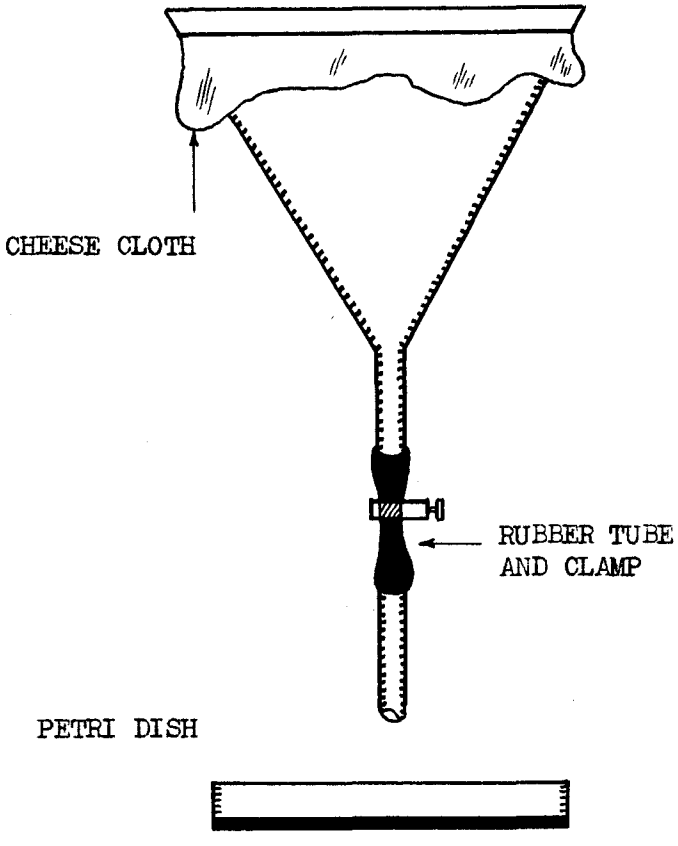

FIGURE III

BAERMANN APPARATUS

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