THE EFFECT OF A COLLOIDAL ANTACID UPON TRICHINELLA SPIRALIS

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CHAPTER I

INTRODUCTION

The parasitic disease, trichinosis, produced by *Trichinella spiralis* (Owen, 1835), has been the subject of numerous investigations.

Early surveys have shown the incidence of human trichinosis in the United States as a whole to be 16 per cent.\(^1\) This high infection rate has placed this country at a focal point in the development of measures concerning eradication of the disease.

More recent studies by Most and Zimmerman have revealed a significant decline in the disease within the population of the United States as a whole and in the state of Iowa, respectively.\(^2,3\)

Better control procedures undoubtedly have effected the dramatic reduction of trichinosis in this country; however investigators conducting research in the problem have failed to find a suitable cure for the disease.

Gould has suggested that the lack of a suitable treatment against the nematode may be associated with two factors: (1) the lack of suitable agents to react directly with the larval and intestinal forms, and (2) the biology of the parasite itself, which permits it to take evasive action by

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quickly penetrating the mucosa of the intestinal tract after infection.\textsuperscript{1} If the intestinal mucosa can be changed by chemical or physical means, the rate of penetration of the intestinal wall by the *Trichinella* larvae may be altered subsequently.

Neo-Gel\textsuperscript{3}, a formulation of a hydrated colloidal tricalcium phosphate and magnesium trisilicate was used in this investigation. The antacid has been demonstrated by clinical and experimental observations to have coating action, and good therapeutic effects in the reduction of gastric hyperacidity.\textsuperscript{2}

The purpose of this investigation was to measure the efficacy of this colloidal antacid upon the intestinal stage and/or the larval production of *Trichinella spiralis* after the drug had been allowed to coat the intestinal tract of the white laboratory mouse.

\textsuperscript{1}Could, op. cit., p. 261.

CHAPTER II

HISTORICAL BACKGROUND

The basic groundwork that clarified early investigations concerning the life history and development of the parasitic nematode, Trichinella spiralis, was formulated in the latter part of the nineteenth century by the German zoologists Leuckart and Virchow. Since that time much advancement has been made in this area of parasitic research; however, there still remains no unified agreement as to the specific pattern of development of Trichinella.

Heller, in a series of ingenious experiments with colloidal sacks suspended within the small intestine of experimental cats, showed that Trichinella larvae could not develop properly unless they were allowed to penetrate and utilize the mucosal lining of the small intestine. He showed that the larvae develop quickly after enzymatic release from their cysts and enlarge into sexually mature adults within two days.

Holding was detected twenty hours post-infection by Hewert-Talswick and Budge. They showed that copulation occurred about four

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3 Ibid.

hours after infection in the white rat, and the larvae to have matured within four days. Further studies by Kreis emphasized that female trichinae undergo four molts, two before copulation, at the second through eighth hour, and twelfth through sixteenth hour post-infection, and an additional two molts after copulation at the forty-eighth and seventy-second hour. The male larvae were reported to have undergone only three molts, two before copulation, which occurs at about the eighteenth hour and once after copulation at the twentieth hour after infection. Gould et al. noted insemination in female trichinae within laboratory rats as early as thirty hours after infection and assumed that this process could occur more than once. The report by Ma and Kingscote showed the final molt to have been completed in rats at thirty-three hours and insemination occurring at the thirty-second hour after infection with excysted larvae. According to Goodshield, Trichinella larvae contained within thirty-ve versa fistulas of white rats were able to mature, reproduce and produce infective larvae; however the intestinal adults were stunted in size and the degree of infection in

1Ibid.  2Ibid.  3Ibid.  4S. F. Gould and others, "Studies on Trichinella spiralis I. Concerning the Time and Site of Insemination of Females with Trichinella spiralis," American Journal of Pathology, XXXI (September-October, 1953), 231-236.  

the host was reduced. Studies by Villella have revealed different results. His experiments with albino rats showed both the male and female trichina larvae to molt four times. The female worm underwent ecdysis at intervals of six, twelve, eighteen, and twenty-four hours and the male trichinas at twelve, eighteen, twenty-four, and thirty hours after infection. According to Khan who studied the post-embryonic development of Trichinella within the white laboratory mouse, excysted male trichinae underwent ecdysis at intervals of ten, seventeen, twenty-four and twenty-nine hours post-infection, whereas female larvae molted at intervals corresponding to twelve, nineteen, twenty-six and thirty-six hours. Definite morphological alterations, both internal and external, accompanied the last three molts in male trichinae and the last two molts in female worms. An extensive search of the muscle phase of this parasite revealed no signs of molting. Recently Berntzen has given support for the decapsulated trichinae larvae to undergo only one molt and this occurring as nearly as eight hours to as late as seventy-two


3 Ibid.


5 Ibid.

6 Ibid.
hours post-infection within the laboratory rat. Although the intestinal molt was not found to occur at any specific time its presence always indicated a period when sexual differentiation was completed. Berntzen further demonstrated that the juvenile larvae molt once within the egg shell producing a second stage larva which migrates into the host's tissues. During the muscular phase within the albino rat Berntzen has shown two additional molts, one on the eleventh day and the other at the fifteenth day post-infection. Berntzen has given support to those researchers who have felt that Trichinella displays a typical life cycle characteristic of most parasitic intestinal worms. Such a life history predisposes two environments for the parasite which results in the parasite becoming infective in one of these during the fourth stage of development.

In vitro studies have added much to the literature concerning the development of Trichinella; however many of these culture systems have served only to maintain the nematodes for an extended period of time and true development with the onset of growth and sexual maturation has not been reported.

Keilty cultured excysted Trichinella in a nutrient salt solution for seven days and noted an increase in their length from 0.6 to

2 Ibid.
3 Ibid., p. 103.
4 Ibid., p. 105.
5 Ibid., p. 71.
1.5 millimeters.\(^1\) Little success was obtained with minced ten day chick embryos and Tyrode's solution by McCoy, although injection of the trichinae directly into living chick embryos did prove slightly successful.\(^2\) Further experiments by McCoy showed that larvae injected into pregnant rat embryos or directly into the horn of the uterus could develop and produce infective larvae.\(^3\) By successfully cultivating the larvae in a foreign environment, McCoy postulated that the occurrence of host food, digestive enzymes, bacteria and their products did not influence or serve for the complete development of the larvae.\(^4\) A subsequent culture attempt for Trichinella which was similar to that of McCoy's earlier work was undertaken by Levin who kept the excysted larvae alive for eleven days in a medium of Tyrode's solution and glucose.\(^5\) His work proved the medium to be a maintenance system and no development was recorded.\(^6\) Weller, who cultured the larvae in roller tube tissue cultures demonstrated that the male and female worms undergo four molts which were not complete.\(^7\) He also found that sexual

\(^{1}\) Gould, loc. cit.


\(^{3}\) Ibid.

\(^{4}\) Ibid.


\(^{6}\) Ibid.

differentiation and the appearance of the vulva and anal papillae occurred after the third incomplete molt. Kim using a modified chick embryo extract and serum medium produced results similar to Weller. The male and female trichinae underwent four molts to sexual maturity; however they all displayed a multiple sheathing effect and eventually died. From a review of such research, Silverman has suggested that the larvae might require no nutrient until the adult stage, and that their growth and development are dependent upon a physico-chemical stimulus. Rogers has also shown that the physical and chemical processes of the intestinal tract "trigger" the hatching of ascarid eggs and the larvae of trichostrongyles. The complexity and dynamic influence of the vertebrate gut and its relationship to intestinal parasites have been emphasized in the comprehensive monograph by Read.

Using a recirculation culture system Berntsen produced male Trichinella with sperm and female larvae with developing ovaries.

1 Ibid.
5 Clark P. Read, Jr., "The Vertebrate Small Intestine as an Environment for Parasitic Helminths," Rice Institute Pamphlet (July, 1920), 77.
Later he added that these larvae underwent only one molt but could be induced to produce many more. These "stimulated" worms displayed the previous multiple sheathing, a reduction in size, females with no embryos, and eventual death.¹ Meerovitch, using roller-tubes with chick embryos and rabbit serum, showed trichinae larvae to undergo development through two stages of incomplete ecdysis.² These larvae eventually died and showed no increase in size.³ In addition Meerovitch showed support for the importance of a gaseous environment consisting of nitrogen or 5 per cent carbon dioxide in nitrogen.⁴ Meerovitch has agreed with Sommerville that the morphological changes induced in each stage of development in some parasitic helminths do not require an environment of high carbon dioxide tension; the changes probably occur by a "triggered" stimulation from carbon dioxide gas or carbonic acid individually or in a combined form.⁵ Bertzen reported the development in vitro of the larvae of Trichinella to mature adults which produced young.⁶ In these experiments he demonstrated only one molt that occurred in the interval of time similar to his prior in vivo

¹Ibid.
³Ibid. ⁴Ibid. ⁵Ibid. ⁶Ibid.
studies. His work showed that a culmination of the gaseous environment, presence of reducing agents, pH, the culture medium and temperature greatly affected the successful cultivation of Trichinella. In his research Berntzen considered a successful culture as one in which multiple sheathing did not occur and those in which growth and sexual maturation developed.

A number of agents have been employed in studies with Trichinella which have altered the development of the parasite. Gould has given a good review of physical factors, immune serums and chemotherapeutic agents studied prior to 1943 while Kagan has presented a comprehensive review of immunological consequences which affect the development of Trichinella. In addition, a recent report of chemotherapeutic agents for parasitic helminths has been surveyed by Standen.

Further research has shown that the proteolytic enzyme, papain, the parasitve chemical, magnesium sulphate, and the parasympathetic

1 Ibid. 2 Ibid. 3 Ibid.
blocking drug, atropine, were ineffective as physical agents in reducing the number of adult trichinae.\textsuperscript{1, 2, 3}

A greater number of larvae were recovered from stressed mice exposed to electrical stimulation, bright light and loud noises.\textsuperscript{4}

Mice treated with antihistamine or antiserotonin drugs harbored more intestinal worms during the seventeenth and twenty-first days after infection than did controls.\textsuperscript{5} In addition studies by Rauss have shown that tranquilizers increase the susceptibility of white rats to infection with \textit{Trichinella}.\textsuperscript{6}

Kozer has shown that mice exposed to thirty-seven degree centigrade temperatures for several hours or subjected to daily muscular work have reduced numbers of encysted larvae when infected with trichinae larva.\textsuperscript{7} According to Chute the decrease in body metabolism

\begin{enumerate}
\item Evelyn A. Rauss, "Effect of Reserpine on \textit{Trichinella spiralis} Infection in Rats," \textit{Experimental Parasitology}, X (June, 1963), 204-251.
\end{enumerate}
associated with hibernating dormice, bats, and hamsters reduces the number and size of Trichinella larvae. 1, 2, 3

Farnesol, cholesterol and insect extract have been used in in vitro studies with Trichinella by Meerovitch. The insect extract containing ecdysone allowed complete molts in cultures that previously produced multiple sheathing. 4 Farnesol inhibited development while cholesterol was found to be important in the development of the parasite. 5 Recent studies concerning digestion procedures carried on in vitro have been reported by Berntzen. 6 He demonstrated that larvae treated after peptic digestion with an additional digestive solution made up of enzymes and salts equivalent to those found in the small intestine would produce greater yields of normal mature adults in culture. 7

The chemotherapeutic drugs, farnosoph, dithiazanine iodide, fluelone, naphthoquinones, piperazine compounds, methyridine have been


5Ibid. 6Berntzen, loc. cit. 7Ibid.
shown to affect the number of adult larvae that remain in the intestine. 1, 2, 3, 4, 5, 6, 7

Riedel using sulphanilamide and sulphamerazine in the diet, noted that the greatest efficacy when the compounds were combined; the larval counts of muscle tissue showed a reduction of 67.1 per cent to 79.9 per cent from that of controls. 8 Further research by Gallicchio has reported that filipin, streptomycin sulphate, patulin,


2Ibid.


4Zimmerman, loc. cit.

5J. Oliver-Gonzalez and E. Beuding, "Reduction in the Number of Adult Trichinella spiralis in Rats after Treatment with Antihistamines," Proceedings of the Society for Experimental Biology and Medicine, LXX (December, 1941), 709.


erdomycin, amicetin, strptolydigin and U-6591 significantly reduce the larvae from the muscle of the host.\(^1\) Mice received a daily dose of the antibiotics twenty-four hours post-infection for fourteen consecutive days.\(^2\) Larsh et al. have shown that a diet supplemented with cadmium oxide reduces the number of adults and larvae of *Trichinella spiralis*.\(^3\) Continued studies with pigs and mice emphasize the prophylactic nature of the drug in reducing the number of larvae recovered from the skeletal muscles.\(^4, 5\)

The organophosphate Neguvon has proved successful in affecting the intestinal, migrating and muscular stages of *Trichinella*. Lamina using white mice given high concentrations of Neguvon in combination with an antidote reported 100 per cent and 87 per cent efficacy in the intestinal and migrating stages, respectively.\(^6\) Zimmerman also using

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\(^2\) Ibid.


Neguvon in combination with an antidote showed that in pigs the drug was effective against both migratory and muscular phases, reducing the trichinae larva by 99 per cent.¹

The chemotherapeutic agent, thiabendazole, given in high doses to mice, killed adult *Trichinella* in the intestine, killed early migratory larvae and either killed or caused the fully developed larvae in the muscle to become non-infective.² Zimmerman has also demonstrated that this drug is effective in reducing trichinae yields in pigs by 99 per cent.³ Aikawa has stressed the use of thiabendazole as a primary treatment in combating human trichinosis; however because of insufficient clinical data he does not indicate that the drug successfully kills the larvae in the muscle tissue of the host.⁴

Neo-Gel, a commercial antacid, was used in this investigation in order to alter the environment within the small intestine of the white laboratory mouse. The experiment was designed to determine if Neo-Gel would have an effect upon the development of *Trichinella* larvae injected into mice per os. The drug produced by Diamond Laboratory,

¹Zimmerman, loc. cit.
³Zimmerman, loc. cit.
Des Moines, Iowa consists of a special formulation of Neojel, a colloidal hydrated tribasic calcium phosphate and a hydrated magnesium trisilicate compound. Neojel was developed by Diamond Laboratory in a search for better methods of concentrating antigenic fractions of cultures of bacterial organisms for the production of vaccines and bacterins. The outstanding property of Neojel that warrants its use as an adjuvant stems from its adsorptive power. Both Neojel and the component magnesium trisilicate exhibit antacid properties; however, when in combination to form the suspension Neo-Gel, the effective action is increased as shown in in vitro studies. Further in vitro studies have shown Neo-Gel to demonstrate a simulated gastric pH of four and above for more than two hours. Clinical studies by Jackson, Figueroa, Kauvar, and Steigmann have supported the relationship between


2Ibid., p. 1.

3E. Jung and others, "The Use of Tricalcium Phosphate for the Adsorption and Concentration of Hemipelas Bacterin," American Journal of Veterinary Research, XXI (September, 1960), 902-903.

4Diamond Laboratory Research Department, op. cit., pp. 7-8.

5Ibid.
Neo-Gel and its effective reduction of gastric hyperacidity.\(^1\), \(^2\), \(^3\), \(^4\)

Neo-Gel's mechanism of action rests partially in its ability to coat the stomach. Studies by the research facilities of Diamond Laboratory have demonstrated by X-ray analysis that preparations of barium sulphate combined with Neo-Gel significantly coats the gastrointestinal tract of the experimental dog.\(^5\)

\(^1\)James E. Jackson and Mark D. Ravreby, "Clinical Experience with Neo-Gel, a New Antacid," *Journal of Iowa State Medical Society*, (June, 1960), 308-309.


\(^5\)Diamond Laboratory Research Department, *op. cit.*, p. 13.
A suspension of Neo-Gel (Lot # 58006), was obtained in twelve ounce bottles from Diamond Laboratory, Des Moines, Iowa. Preliminary experiments were run to determine the intestinal emptying time and coating action of Neo-Gel within the small intestine of the white laboratory mouse.

A strain of male mice three months old that was maintained at Drake University, Des Moines, Iowa was used in this preliminary experiment. The white laboratory mice were divided into four groups of three mice each and treated in the following manner. Mice in control Group I were individually anesthetized with ether (Merck, Rahway, New Jersey) by placing them in a closed glass etherizing chamber. When the mice showed signs of extended breathing they were removed from the etherizing jar and held by the nape of the neck with the thumb and forefinger while at the same time grasping the tail with the little finger of the same hand. The tip of a micro-pipette (Scientific Products, Inc., Evanston, Illinois, # F-321C-1) was introduced into the mouth and esophageal tract of the mouse and 0.5 cubic centimeters of water in combination with blue food coloring (Tone's, Des Moines, Iowa) was introduced. The remaining experimental mice in Groups II, III, and IV were injected in a similar manner with
0.5 cubic centimeters of Neo-Gel thoroughly mixed with the food coloring. At intervals of three, six, and twelve hours, respectively, the three mice from each experimental group were sacrificed along with one mouse from the control group. The gastro-intestinal tracts were removed from the sacrificed animals and were placed in a dissecting pan. The stomachs and small intestines of the mice were slit longitudinally with a small scalpel exposing the mucosal surface. Measurements of the movement of Neo-Gel in combination with the food coloring and appraisal of the coating action were made with the aid of a Bausch and Lomb stereoscopic microscope using a 10X ocular and a variable high power objective.

A strain of Trichinella spiralis originally obtained from W. R. Zimmerman of Iowa State University, Ames, Iowa was used in this investigation. Trichinella larvae that were to be used experimentally were digested artificially from stock cultures of infected white mice maintained at Drake University, Des Moines, Iowa. The stock cultures of the parasite were renewed periodically at intervals not exceeding two months and the larvae used in each experimental run were obtained from mice infected approximately one month prior to the time of digestion of the infected animal.

Infected larvae used in conjunction with the set of experimental runs were obtained by a modified digestion method described by Schell.\(^1\) The trichinized mice were sacrificed by vertebral

disjuncture, the tail, feet, and head removed, carcass skinned and
eviscerated. The remaining musculature was cut into small pieces
and placed into a Waring Blender (A. S. Aloe Company, St. Louis,
Missouri). Into the blender was added 150 milliliters of a solution
consisting of 0.67 per cent pepsin powder (Fisher Scientific Company,
Fair Lawn, New Jersey), 1 per cent concentrated hydrochloric acid
(Callenckrodt Chemical Works, New York, New York), and tap water warmed
to a temperature of 37° Centigrade. The muscle tissue and digestion
fluid were blended for approximately one minute at the medium speed
setting, and then transferred to a five hundred milliliter (Pyrex)
screw top erlenmeyer flask. An additional 150 milliliters of digestion
fluid was added to the Waring Blender, the remaining muscle tissue
removed and transferred to the erlenmeyer flask. The flask containing
the digestion mixture and homogenized muscle was incubated at 37°
Centigrade. At the end of four hours, two hundred milliliters of the
digestion fluid were removed by placing the tube of an aspirator
(Emberth, B. S. A.) under the surface layer of the liquid. The
remaining carcass and larvae were washed with three hundred milliliters
of water, the water poured into and agitated within the erlenmeyer flask.
The excised larvae were allowed ten minutes to settle to the bottom
of the flask before the procedure was continued. Aspiration and wash-
ing were repeated until the digestion solution was clear to light pink
in color. The remaining digestion fluid was poured through four layers
of cheese cloth suspended on a wire screen (Grid of thirty-two lines per
square inch) which was placed in a four-inch glass funnel. A piece of
rubber tubing was inserted on the stem of the funnel to which was added a pinch cock. This modified Baermann apparatus containing the larval fluid was placed in a 37° Centigrade incubator and the suspension of larvae allowed to migrate and concentrate at the base of the funnel. At the end of one hour the concentrated larvae were drawn off from the Baermann apparatus into a 150 milliliter erlenmeyer flask. A two-inch long polyethylene stirring device was placed within the flask which was set upon a magnetic stirrer (Magnestir # 1250 Labline, Inc., Chicago, Illinois). The larval solution was stirred at a constant rate and 0.5 cubic centimeter aliquots were withdrawn with a micro-pipette and placed within the depressions of a spot plate. The number of larvae present was determined with the aid of an Adams Laboratory Counter (Clay-Adams, Inc., New York, New York). The larval suspension was either concentrated or diluted with warm tap water in order to obtain aliquots of approximately sixty trichina larvae. The total number of larvae were counted from ten aliquot samples before and after an experimental run and the average number of larvae injected per 0.5 cubic centimeter determined for each experimental run.

A strain of white male mice three months of age or older were obtained from the National Laboratory Animal Company, Creve Coeur, Missouri. Three experimental runs were designed with mice divided into five groups of seven or eight mice each and one control group consisting of two mice. Two experimental groups were either infected with Trichinella larvae one hour before or one hour after the initial
injection of Neo-Gel. The remaining experimental group was infected with larvae treated in vitro with the drug. After the first injection of Neo-Gel the drug was repeatedly injected in two experimental groups and two control groups every six hours for the first thirty hours post-infection and then every twelve hours for an additional ninety-six hours. Group I served as an infected control to which a placebo of tap water was administered following the initial infection with Trichinella larvae. Two mice in Group II were maintained as uninfected controls to which Neo-Gel was administered at the specified intervals. Group VI served as a stress control. This group of mice were not treated with Neo-Gel but received only an initial injection of Trichinella larvae. Groups III and IV served as experimental mice and received, respectively, Neo-Gel one hour after and one hour before the infective dose of larvae. Subsequent injections of the drug were given this group at the previously specified intervals. Group V was maintained as an in vitro study of the drug. The standard aliquots of 0.5 cubic centimeters of larval suspension were treated within individual spot plate depressions, each with four drops of Neo-Gel applied from a medicine dropper. The suspension of larvae treated with the Neo-Gel were kept in the spot plate depressions for approximately fifteen minutes at the end of which time they were injected into the mice. The schedule of injections for experimental and control groups has been tabulated in Table I. All groups of mice were housed in individual plastic cages with metal tops and were allowed food (Purina Chow) and water ad libitum.
### TABLE I

**SCHEDULE OF NEO-GEL INJECTIONS FOR EXPERIMENTAL AND CONTROL MICE INFECTED WITH TRICHINELLA SPERATAE**

<table>
<thead>
<tr>
<th>Initial injection schedule for white mice</th>
<th>Continued injection schedule for white mice</th>
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<tbody>
<tr>
<td>Starting time: One hour later</td>
<td>Every six hours for the first thirty hours,</td>
</tr>
<tr>
<td></td>
<td>then every twelve hours for an additional ninety-six hours</td>
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<table>
<thead>
<tr>
<th>Group number</th>
<th>Injection procedure</th>
<th>Injection number</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>I</td>
<td>Water</td>
</tr>
<tr>
<td>II</td>
<td>Trichinella</td>
<td>II</td>
<td>Neo-Gel</td>
</tr>
<tr>
<td>III</td>
<td>Trichinella</td>
<td>III</td>
<td>Neo-Gel</td>
</tr>
<tr>
<td>IV</td>
<td>Neo-Gel</td>
<td>IV</td>
<td>Trichinella</td>
</tr>
<tr>
<td>V</td>
<td>Trichinella</td>
<td>V</td>
<td>--</td>
</tr>
<tr>
<td>VI</td>
<td>Trichinella</td>
<td>VI</td>
<td>--</td>
</tr>
</tbody>
</table>

**in vitro**

**Stress control**
The procedure for injecting the mice with Neo-Gel in the experimental and control groups was similar to the technique used to introduce the infective larvae in the preliminary experiment and in the experimental runs except for two points. Before each injection schedule the suspension of Neo-Gel was thoroughly shaken for two minutes and then poured into a fifty milliliter beaker. In addition it also became necessary to lubricate the tip of the micro-pipette with a cloth saturated with water before each mouse was injected with the drug. The film of water on the pipette prevented any excess drug from drying on the outside and allowed for easier penetration into the esophageal tract of the host organism.

After thirty days post-infection the six groups of mice were sacrificed by vertebral disjuncture. Each mouse was placed on its dorsal side within a dissecting pan and both the front and back limbs pinned to the pan. An incision along the midline on the ventral surface was made from the posterior end of the mouse to a centimeter anterior to the diaphragm. Midway on this longitudinal incision a transverse incision was made extending to each side of the dorsal aspect of the mouse. By clipping off the xiphoïd process close to the rib cage a small hole was left in the upper border of the diaphragm. Inserting surgical scissors in this region and at the same time pulling the diaphragm away from the body wall with pointed forceps the muscle tissue could be cut away from both sides of the mouse. Before cutting the remaining dorsal segment of the diaphragm from the body wall all
internal viscera and adhering tissues were removed from the diaphragm. Diaphragms from each group of mice were washed in cold tap water and placed in a labeled beaker of water under refrigeration. Diaphragms within their respective beakers of tap water were removed from refrigeration approximately twenty-four hours before they were to be counted and left exposed to the air. A slight softening of the tissues had occurred to the extent that the diaphragms could be easily compressed between two standard microscope slides with metal paper clamps (Hunt Clip #0, Camden, New Jersey). Only a few encysted larvae could be detected within the beakers of water and therefore one can assume that there was a negligible loss of trichinae from the diaphragms.

The diaphragm press was placed upon the mechanical stage of a compound microscope (Spencer, 100X) and the larvae counted with the aid of a Whipple eye piece using a 10X objective. The diaphragms were counted from top to bottom and the number of larvae seen within the gridded scale were recorded. The excysted larvae were counted twice from top to bottom, sweeping the field of view, and from bottom to top with each diaphragm. Data from individual counts of diaphragms were averaged and recorded. Diaphragms from each group and experimental run were stored and fixed in a 10 per cent solution of formalin.
An experimental investigation involving *Trichinella spiralis* was designed to determine if the coating action of Neo-Gel, a colloidal antacid, would have a physical or chemical effect upon the development of the intestinal stage and/or larval production of the parasite within the small intestine of the white laboratory mouse.

Preliminary studies with Neo-Gel were undertaken to determine the intestinal emptying time and relative coating action of the drug within the small intestine. Experimental mice were injected orally with Neo-Gel in combination with food coloring and sacrificed at intervals of three, six, and twelve hours. Control mice were similarly injected and sacrificed; however these mice were given water in combination with the food coloring. The stomachs and the small and large intestines of the mice were removed and examined using a 10X ocular and 20X objective of a Mauch and Leeb stereoscopic microscope.

Stock mice infected with *Trichinella* larvae were artificially digested with a solution of pepsin and hydrochloric acid. The infective larvae, which were recovered from the musculature of the mice, were concentrated and cleaned of debris by means of a modified Sauer- mann apparatus. The *Trichinella* larvae were diluted with warm tap water to obtain aliquots of approximately sixty larvae per 0.5 cubic centimeters of solution for each experimental run as indicated in
Table II. The infective larvae were injected orally into groups of control and experimental mice with a micro-pipette. A volume of 0.5 cubic centimeters of Neo-Gel was also injected orally into groups of control and experimental mice in a similar manner. Three experimental runs of six groups of seven or eight mice each and one group with two mice were used in this study. These groups of mice served as experimentals and controls in the investigation and their injection schedules have been summarized in Table I.

### TABLE II

<table>
<thead>
<tr>
<th>Experimental run</th>
<th>Mean number counted before injections</th>
<th>Range</th>
<th>Mean number counted after injections</th>
<th>Range</th>
<th>Mean of both counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>74.3</td>
<td>72-63</td>
<td>63.2</td>
<td>57-69</td>
<td>68.8</td>
</tr>
<tr>
<td>II</td>
<td>66.1</td>
<td>59-79</td>
<td>66.6</td>
<td>55-72</td>
<td>66.3</td>
</tr>
<tr>
<td>III</td>
<td>55.3</td>
<td>45-67</td>
<td>55.8</td>
<td>44-66</td>
<td>55.6</td>
</tr>
</tbody>
</table>

After a period of thirty days the mice were sacrificed and their diaphragms removed. The diaphragms were pressed between two standard glass microscope slides with metal clamps and placed upon
the stage of a compound microscope. The number of larvae encysted in
the musculature of the diaphragms was observed and recorded for each
mouse in the experimental and control groups. The average number of
larvae and the range of the individual counts was calculated for each
group of mice. Because the normal curve represented by small samples
makes a difference appear more significant, an estimated standard
deviation instead of a standard deviation that approaches an infinite
sample was determined for each group of mice. The number of degrees
of freedom was used as the divisor in calculating the estimated stan­
dard deviation instead of dividing by the total number of measure­
ments. A "Student's" T evaluation was applied to the data in order
to compare the means between control Group I and the experimental
mice, and percentage points for each experimental group were determined
from a statistical table to show the probability of obtaining the
observed difference by chance. The results of the three experimental
runs are tabulated in Tables III, IV, and V.

Results from the preliminary experiment have shown that mice
autopsied at the three-hour interval after injection have approximately

1Ronald Rainland, The Treatment of Clinical and Laboratory Data
(London: Oliver and Boyd, 1938), pp. 35-36.

2Ibid.

3Alvin W. Lewis, Biostatistics (New York: Reinhold Corporation,
1966), pp. 52-62.
an even distribution of Neo-Gel throughout the gastro-intestinal tract except in the region of the stomach and caecum where the drug was pooled. The control mouse sacrificed at the same interval of time showed a more rapid emptying of the contents of the gut. The tracer was noticeable in the lower one-third of the small intestine, in the caecum and in the colon. The control mouse also showed a heavy concentration of the tracer pooled within the caecum. The experimental mice sacrificed at the six-hour interval post-injection demonstrated Neo-Gel to be concentrated in the caecum and colon of the gastro-intestinal tract. No noticeable tracer was observed in the stomach or small intestine. The control mouse autopsied at the sixth hour after injection showed no indication of the tracer. Their remaining experimental mice and the control mouse sacrificed at the twelfth hour after injection also showed no signs of the tracer. In those instances where Neo-Gel in combination with the tracer was found within the intestine a coating effect was demonstrated upon the mucosal surface. This preliminary experiment indicated that Neo-Gel could be retained within portions of the small intestine for approximately three hours and that the suspension would coat the mucosal surface of the intestinal tract.

It can be observed from the data obtained in experimental run number I, that Neo-Gel administered to Group IV one hour before the infective dose of Trichinella reduced the encysted larvae in the diaphragm. The encysted larvae in mouse diaphragms of experimental Groups
III and V have shown a slight increase and decrease respectively from the mean larval number in control Group I. The percentage points computed from T values of these groups of experimental mice exceed the 5 per cent confidence level. Control mice in Group I demonstrated excessive fighting and at the time of sacrifice all mice were badly chewed-up. The response of mice in control Group II has indicated that the dosage of Neo-Gel in this experimental run was well tolerated.

Results from experimental run number II have shown a reduction in the mean number of encysted larvae within the diaphragms of all experimental mice when compared to the control, Group I. Mice in experimental Group IV showed the greatest reduction in larvae within the diaphragms when compared to control Group I. A T value of 5.71 corresponding to percentage points of under 0.1 per cent shows a highly significant response well under the 5 per cent confidence level. In a similar manner Groups III and V indicate a significant reduction in encysted larval numbers within the diaphragm as compared to the mean number of the control Group I. These experimental groups of mice also have computed percentage points under a 5 per cent confidence value. A considerable amount of stress indicated by the low value of encysted larvae within the diaphragms of control Group VI indicates the effect of the injections upon the other control and experimental groups.

After two weeks post-infection, mice in control Group I began to die, and at the end of three weeks, two physically sluggish mice remained within this group. Three dead mice recovered from Group I had
### TABLE III

**NUMBER OF TRICHINELLA SPIRALIS LARVAE IN MOUSE DIAPHRAGMS**  
**EXPERIMENTAL RUN NUMBER II; CONTROLS AND MICE INJECTED**  
**WITH 0.5 CUBIC CENTIMETERS OF NEO-GEL AT SPECIFIC INTERVALS FOR ONE-HUNDRED AND TWENTY-SIX HOURS POST-INFECTION**

<table>
<thead>
<tr>
<th>Group number</th>
<th>Number of mice per group</th>
<th>Range</th>
<th>Mean control or experimental</th>
<th>Estimated standard deviation</th>
<th>T value and percentage points</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
<td>5</td>
<td>339-549</td>
<td>467</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II Control</td>
<td>2</td>
<td>--</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III Neo-Gel injected</td>
<td>7</td>
<td>332-998</td>
<td>474</td>
<td>219 T = 0.056 P &gt; 50%</td>
<td></td>
</tr>
<tr>
<td>IV Neo-Gel injected</td>
<td>8</td>
<td>146-392</td>
<td>236</td>
<td>60 T = 3.60 P &gt; 0.1% not &gt; 0.5%</td>
<td></td>
</tr>
<tr>
<td>V Neo-Gel in vitro</td>
<td>6</td>
<td>198-670</td>
<td>393</td>
<td>222 T = 0.66 P &gt; 50%</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE IV

**NUMBER OF TRICHINELLA SPIRALIS LARVAE IN MOUSE DIAPHRAGMS EXPERIMENTAL RIB NUMBER II: CONTROLS AND MICE INJECTED WITH 0.5 CUBIC CENTIMETERS OF NEO-CEP AT SPECIFIC INTERVALS FOR ONE-HUNDRED AND TWENTY-SIX HOURS POST-INFECTION**

<table>
<thead>
<tr>
<th>Group number</th>
<th>Number of mice per group</th>
<th>Range</th>
<th>Mean control or experimental</th>
<th>Estimated standard deviation</th>
<th>T value and percentage points</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
<td>4</td>
<td>2143-3377</td>
<td>2554</td>
<td>574</td>
<td></td>
</tr>
<tr>
<td>II Control</td>
<td>2</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
</tbody>
</table>
| III Neo-Cel injected | 6 | 740-1572 | 1197                        | 459                          | $T = 3.34$  
P = $0.1\%$ not $> 2.5\%$ |
| IV Neo-Cel injected | 7 | 711-2342 | 1211                        | 574                          | $T = 5.71$                        
P = not $> 0.1\%$ |
| V Neo-Cel in vitro | 7 | 577-1748 | 1023                        | 402                          | $T = 1.63$                        
P = $0.1\%$ not $> 0.5\%$ |
| VI Stress control | 6 | 746-1116 | 816                         | 175                          | $T = 6.16$                        
P = not $> 0.1\%$ |
### TABLE V

**NUMBER OF TRICHINELLA SPHINX**

**EXPERIMENTAL RUN NUMBER III: CONTROLS AND MICE INJECTED WITH 0.5 CUBIC CENTIMETERS OF NEO-CHEL AT SPECIFIC INTERVALS FOR ONE-HUNDRED AND TWENTY-SIX HOURS POST-INFECTION**

<table>
<thead>
<tr>
<th>Group number</th>
<th>Number of mice per group</th>
<th>Range</th>
<th>Mean control or experimental</th>
<th>Estimated standard deviation</th>
<th>T value and percentage points</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
<td>7</td>
<td>1387-2850</td>
<td>1779</td>
<td>517</td>
<td></td>
</tr>
<tr>
<td>II Control</td>
<td>2</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>III Neo-Cel injected</td>
<td>6</td>
<td>941-2078</td>
<td>1439</td>
<td>419</td>
<td>T = 1.14, P &gt; 25% not &gt; 50%</td>
</tr>
<tr>
<td>IV Neo-Cel injected</td>
<td>6</td>
<td>336-1454</td>
<td>1060</td>
<td>666</td>
<td>T = 1.93, P &gt; 5% not &gt; 10%</td>
</tr>
<tr>
<td>V Neo-Cel in vitro</td>
<td>7</td>
<td>613-2200</td>
<td>1382</td>
<td>515</td>
<td>T = 1.43, P &gt; 10% not &gt; 25%</td>
</tr>
<tr>
<td>VI Stress control</td>
<td>6</td>
<td>947-1888</td>
<td>1267</td>
<td>314</td>
<td>T = 1.74, P &gt; 10% not &gt; 25%</td>
</tr>
</tbody>
</table>
deteriorated too much to make a larval determination from the diaphragm muscle. The deaths of the mice that occurred in this control group suggest a large larval burden within the host, as contrasted to those mice in the other experimental and control groups. The condition of mice in control Group II indicated that the dosage of Neo-Gel was well tolerated.

All of the groups of mice in experimental run number III showed a reduction in the mean number of larvae encysted in the diaphragms when compared with the mean larval numbers of control Group I. Of these experimental groups only Group IV showed a difference between the encysted larvae and that of the control that may be called slightly significant. The F value expressed for Group IV was 1.93 with percentage points of not greater than 10 per cent. Although these percentage points exceed the accepted confidence level of 5 per cent, Group IV, judged relative to the other groups of experimental mice, suggest that this percentage is significant in this experimental run. It appears that too much control Group IV, in which stress was placed upon the experimental and control mice by the injections did not have as great an effect as determined by the number of encysted larvae in this control run. It can be seen that the control Group II again tolerated the dosage of Neo-Gel well, in this experimental run.

An over-all view of the three experimental runs has shown that larvae encysted in the diaphragms of mice in all experimental groups except one showed a decrease in the number of Trichinella larvae when
compared to control Groups I. Of the various experimental groups studied, number IV repeatedly showed a significant reduction in encysted diaphragm larvae. In addition larvae encysted in the diaphragms of Groups III and V proved to be significantly lower in number in experimental run number II. In vitro studies showed that the drug has somewhat of an effect directly upon the Trichinella larvae when employing the procedure previously discussed to treat the larvae.

The data from these experiments suggested that a substantial difference occurred in the reduction of larvae within the diaphragms of experimental mice when the initial injection of Neo-Gel was varied only one hour before the infective dose of Trichinella larvae was administered. It was evident that pre-treatment of the environment within the gastro-intestinal tract before Trichinella larvae were introduced led to a greater and repeated alteration in the normal development of the parasite as opposed to those studies in which the Trichinella larvae were introduced into a normal environment and later exposed to Neo-Gel.

It is important to note that the preliminary experiment demonstrated that to continually keep the environment of the gastro-intestinal tract modified with Neo-Gel, one would have to inject the drug approximately every three hours. It was inconvenient to design an experiment whereby the intestinal tract was continually altered with Neo-Gel; therefore the initial injection of Trichinella larvae into a pre-treated environment becomes more important.
One cannot definitely state that Neo-Gel created a physical barrier for the proper penetration of the larvae into the mucosa of the small intestine; however it was shown that the most critical period associated with proper development in the intestine coated with Neo-Gel was within the first hour post-infection when *Trichinella* larvae are attempting to establish themselves within this mucosal lining.

Further studies are necessary to determine the effect of Neo-Gel upon *Trichinella* larvae *in vitro* when one alters the treatment time outside of the host organism. It would also be necessary to determine if the buffering capacity and the subsequent increased pH induced by Neo-Gel within the intestine had an effect upon the *Trichinella* larvae *in vivo*. Additional information should be gathered to establish the time and the specific stage of *Trichinella* larvae that was affected in this study.
CHAPTER V

SUMMARY

The effect of a colloidal antacid, Neo-Gel, upon the larvae of Trichinella spiralis within the small intestine of the white laboratory mouse was investigated. A suspension of this drug was shown to demonstrate a coating effect upon the gastro-intestinal tract of albino mice for an extended period of time. Neo-Gel was administered to groups of experimental and control mice per os in order to alter the normal state of the intestinal mucosa and the environment within the small intestine. By changing the chemical or physical composition of the mucosa of the host organism, the penetration of Trichinella larvae might be altered and the subsequent development of the parasite modified.

The effect of Neo-Gel upon the Trichinella larvae was measured by counting the number of encysted diaphragm larvae within control and experimental mice and comparing the collected data by statistical analysis.

Experimental mice were divided into three groups. Group III received 0.5 cubic centimeters of Neo-Gel one hour after the initial infective dose of Trichinella larvae. Group IV received 0.5 cubic centimeters of Neo-Gel one hour before the infective dose of larvae. Group V was injected with larvae pre-treated with Neo-Gel in vitro.
All experimental groups except number V were repeatedly injected with Neo-Gel at intervals of six hours for thirty hours post-infection and then at intervals of twelve hours for ninety-six additional hours.

The results of three experimental runs have shown a consistent reduction of the mean number of encysted larvae within the diaphragms of experimental groups of mice when compared to the mean number of encysted larvae within the diaphragms of controls. Neo-Gel administered one hour before the infective dose of Trichinella larvae indicated a repeatable and significant reduction in encysted diaphragm larvae. Experimental Groups III and V also showed significant reductions in diaphragm larvae when compared to control groups; however these results could not be repeated in each experimental run.

It is to be suggested from the results of this investigation that when Neo-Gel was allowed to pre-treat the gastro-intestinal tract of the white mouse before administration of Trichinella larvae, significant alteration in the normal development of the parasite occurred.
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BIBLIOGRAPHY

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D. UNPUBLISHED MATERIALS
