1963

The Immunology and Serology of Various Strongyloides Species.

Stanley Herbert Abadie

Louisiana State University and Agricultural & Mechanical College

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Doctor of Philosophy

in
The Department of Tropical Medicine
and Medical Parasitology

School of Medicine

by

Stanley H. Abadie
B.S., Loyola University, 1955
M.S., Louisiana State University, 1958
June, 1963
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ABSTRACT

The comparative immunology and serology of *S. ratti* in rats, *S. fulleborni* in monkeys, *S. myopotami* in nutria, and *S. stercoralis* in man has been studied utilizing agar gel diffusion tests, paper chromatography and electrophoresis. Polysaccharide antigens were prepared from the various species. Blood samples were collected from infected animals and from patients. The sera from infected patients were obtained before, during and after therapy. Control blood samples were collected from patients with numerous negative stool examinations and duodenal drainages. In agar gel diffusion studies, *S. ratti* antigen and antisera produced a single band in 18 hours. In the heterologous systems, *S. ratti* antigen reacted with *S. stercoralis* and *S. fulleborni* antisera. This reaction was not as marked as with the homologous antisera. There was no reaction with *S. myopotami* antisera. *S. fulleborni* antigen and antisera produced two bands in 18 hours. This antigen reacted to a lesser degree with *S. stercoralis* and *S. myopotami* antisera but not with *S. ratti* antisera. *S. myopotami* antigen and antisera reacted after 72 hours. This antigen also reacted after 72 hours with 15 of the 60 *S. stercoralis* antisera tested. *S. ratti* and *S. fulleborni* antisera did not react with the *S. myopotami* antigen. The three antigens—rat, monkey, and nutria—did not react with sera from patients infected with other helminths (*Ascaris lumbricoides*, *Trichuris trichiura* and hookworm) or with sera from patients with negative stools and duodenal drainages. Chromatographic studies revealed one fraction in each of the three antigens. Their Rg values showed *S. ratti* antigen at 105, which approximates the value of viii.
fructose \(10^4\); \textit{S. fulleborni} 64, which is the same as glucosamine; \textit{S. myopotami} 62, which is between trehalose (60) and glucosamine (64).

Electrophoretic patterns of the various serum samples were run before and after absorption with an excess of homologous and heterologous antigens. The largest reduction of gamma globulin seen in the human sera was after absorption with \textit{S. fulleborni} antigen (1.2 cm.). \textit{S. ratti} antiserum produced less reduction (0.4 cm.) and \textit{S. myopotami} antigen no reduction. The homologous system of \textit{S. fulleborni} antigen and antiserum showed a reduction in gamma globulin of 1.9 cm. The heterologous systems of \textit{S. fulleborni} antiserum and \textit{S. ratti} antigen showed a reduction of 1.3 cm. while the \textit{S. myopotami} antigen reduced the gamma globulin 0.7 cm. The homologous system of \textit{S. myopotami} antiserum and antigen showed a reduction in gamma globulin of 0.2 cm. The heterologous system of \textit{S. myopotami} antiserum and \textit{S. fulleborni} antigen showed a reduction of 0.1 cm., while with the \textit{S. ratti} antigen there was no reduction. The results of the absorption studies closely parallel those of the precipitin tests. From the results of the combined studies the relationships of the various species may be determined. With the exception of the nutria strongyle, \textit{S. myopotami}, the various species appear closely related, with \textit{S. stercoralis} and \textit{S. fulleborni} exhibiting the closest relationship. A relationship between \textit{S. ratti} and \textit{S. fulleborni} was demonstrated; however, both are more closely related to \textit{S. stercoralis} than to each other. \textit{S. myopotami} had the least relationship to the others, showing a very slight relationship to only \textit{S. fulleborni}.
INTRODUCTION

Although the immunology of parasitic diseases has been studied for many years, the knowledge in this field is far behind that of other areas. The primary reason for this lag has been the difficulty in culturing helminths axenically. Therefore, without a ready source of antigen from human parasites, related animal species have been used for obtaining antigenic materials. Another reason for the lack of progress has been the ease with which most common helminth parasites may be diagnosed by routine and/or special stool technics. The development of immunological methods of diagnosis was therefore not urgently pursued. Unlike some of the other helminth diseases, strongyloidiasis is not as readily diagnosed by routine stool examination. Jones and Abadie (1954) showed that only 72 percent of a series of 100 cases of strongyloidiasis could be diagnosed by stool examination alone. In order to achieve a diagnostic rate of 100 percent, it was necessary to resort to the use of duodenal drainage as a supplementary measure. This is a tedious and time-consuming procedure. Thus, an additional diagnostic tool for the diagnosis of this parasitic infection seems indicated.

Although species of Strongyloides have been described from numerous animal hosts, their morphology is essentially similar. The classification of species has been based primarily on host specificity. An analysis based on antigenic, chromatographic and electrophoretic studies of certain species of Strongyloides may help to enhance our knowledge of this genus.

Fülleborn (1926) reported the first immunological method for the diagnosis of strongyloidiasis. He collected filariform larvae from
stool cultures of *Strongyloides stercoralis*, washed them in a 1:1000 bichloride of mercury solution and dried them at room temperature. The larvae were then ground in a mortar and the powder stored in glass bottles, for use in scratch testing on the arm. Eleven cases were tested, ten of which had positive stool findings, while the remaining one was a doubtful positive. In these eleven cases, he reported four one-plus reactions and seven two-and-three-plus reactions. Twenty-eight patients who did not harbor *S. stercoralis* but had previous histories of other nematode infections were skin tested. All 28 gave negative results. Although the duration of a positive reaction was unknown, he concluded that the skin test was a useful diagnostic technic.

Sheldon (1939) demonstrated that rats could be successfully immunized against infection with *S. ratti* through serial injections of heat-killed filariform larvae of the same species. Rats inoculated with filariform larvae of a related species, *S. stercoralis*, did not acquire immunity against *S. ratti*. Sheldon reported this as a demonstration of specificity of immunity.

Lawler (1940) using the blood plasma of rats immune to *S. ratti* was able to protect significantly other rats against infection. The serum was administered either before or after the animals were exposed. *In vitro* studies indicated that the protective value of the serum might be due in part to its ability to precipitate the metabolic products of the filariform larvae. Sato (1933) employed a test in which filariform larvae of *Strongyloides* sp. were agglutinated when placed in immune human serum.

Brannon and Faust (1949) prepared more specific antigens for use
in the diagnosis of strongyloidiasis. They collected fecal material from chimpanzees naturally infected with a species of *Strongyloides* (thought to be *S. fulleborni*). The fecal material was mixed with animal charcoal powder and cultured in petri dishes. The larvae were collected from the tops of the petri dishes and washed in a 0.425 percent salt solution containing 0.5 percent phenol. Two methods were used to prepare antigens. In one the larvae were crushed in a ball mill, extracted in sterile saline and dried. In the other method, larvae were ground with sterile emery powder, suspended in Coca's solution, extracted at room temperature for 24 hrs., then at 37°C for 24 hrs. and dried. Bacterial antigens were made from a mixed bacterial flora obtained from the fecal cultures and served as controls. The intradermal and the precipitin tests were employed using the antigens. Twenty-five patients with a diagnosis of strongyloidiasis confirmed by stool examination gave both positive intradermal and precipitin reactions. Four patients who previously harbored *S. stercoralis* were also tested. All four had positive intradermal tests and three had positive precipitin tests. Results were presented also of tests on 108 patients, without previous history of strongyloidiasis. Forty-one gave positive reactions to the *Strongyloides* sp. antigen. These reactions were recorded as doubtful or weak. From these data the authors concluded that the test provided a reliable index of infection. Since the length of time the test remains positive after presumed cure could not be determined, they concluded that the value of the test in these cases was not clear. However, the authors felt that the precipitin test indicated the presence of specific antibody.

Pellegrino, Chaia and Memoria (1961) prepared antigens from
filariform larvae of *S. ratti*. The larvae were collected from stool cultures. They were concentrated by sedimentation and then lyophilized. Coca's solution was added to this material in a flask with glass beads. The mixture was allowed to extract at 4-6°C for three days with frequent agitation. The material was then centrifuged and the resultant supernatant fluid used as an antigen for intradermal testing. The best results were obtained employing a 1:1000 dilution. A papule of 1.0 cm.$^2$ or more was observed in 93.3 percent of the reactions. In higher concentration, 1:100,000, the papules were the same size as the controls using Coca's solution only. In eight negative controls (with five negative stool examinations) in which a 1:1000 dilution of antigen was used, the reactions were all smaller than 0.9 cm.$^2$ and averaged less than 0.47 cm.$^2$. The authors concluded that a significant statistical difference was established and the test was useful for the diagnosis of human strongyloidiasis.

Chaia (1962) studied the intradermal test, using *S. ratti* filariform larvae as antigen. It was his stated objective to evaluate the test as a diagnostic tool for the diagnosis of human strongyloidiasis. The antigen was prepared in the same manner as reported by Pellegrino, Chaia and Memoria (1961). The sites of injection were the flexor surface of the forearm and the upper part of the back. Antigen in 0.05 ml. amounts with 40 micrograms of nitrogen per ml. was used. The reaction was read in 15 minutes. It was concluded that a wheal area equal to 1.2 cm.$^2$ or larger could be considered positive. The patients' back proved the best site for injection. Tests on patients harboring *Ascaris lumbricoides*, hookworm, *Trichuris trichiura*, *Schistosoma mansoni*, *Enterobius vermicularis* and *Taeinia* sp. showed no cross-reactions. Patients treated with
dithiazanine and with negative stools had positive reactions up to one year later. Antigens stored in the refrigerator for six months retained their activity. The author stated that these tests should not be used in epidemiological surveys. In only 80 percent of the positive cases studied in surveys was there agreement between both stool findings and the intradermal test. However, it was felt that the test would be useful in diagnosing individual cases.

Numerous other parasites have been studied immunologically utilizing purified fractionated antigens. Some examples follow. *Trichinella spiralis* has been widely studied. Flury (1913) noted the presence of glucoprotein in the larvae of *T. spiralis*. Melcher (1943) initiated an investigation designed to isolate and identify the chemical fractions of the larvae of *T. spiralis* which were immunologically active. Larvae obtained from infected hogs were washed and lyophilized to make the proteins less subject to denaturation. Four fractions were isolated and tested: the acid-insoluble protein, acid-soluble protein fraction, alkaline extract and polysaccharide. The acid-soluble protein fraction consisted of three electrophoretic components. This fraction produced positive skin and precipitin reactions in infected rabbits. It was a complete antigen capable of inducing and reacting with antibodies produced in rabbits. The antibodies did not cross-react with antigenic material from *Ascaris* sp. adults. The polysaccharide fraction gave positive precipitin reactions but did not give positive skin reactions.

Sarles (1938) working with *Nippostrongylus muris*, a rat nematode, demonstrated the formation of oral and anal precipitates in living larvae when they were placed in immune serum. He felt that these precipitates
were caused by antibodies produced against the secretions and excretions of the worms in the host animal. He set forth the hypothesis that the "formation of oral and intestinal precipitates precedes and appears responsible for the inhibition of the activity and development of the parasite." Other workers who studied this reaction were Oliver-González (1940), Roth (1941) and Mauss (1941) with *T. spiralis*; Otto (1940) with *Ancylostoma caninum*; Lawler (1940) with *S. ratti*; Otto et al. (1942) with *Hecator americanus*; Oliver-González (1943) with *A. lumbricoides*; Smith (1946) with *Trichosomoides crassicauda*; Sadun (1949) with *Ascardia galli*; and Smith (1949) with *Heterakis spumosa*. They all agreed that antibodies produced against the secretions and excretions of the worms were protective. However, none of the workers was successful in duplicating this reaction by using the serum from animals injected with the secretions and excretions. Also, protection was not afforded by the injection of secretions and excretions obtained from these worms.

Thorson (1953), using *Hippoboscyulus muris* larvae, explored the role of secretions and excretions further. He prepared fractions of filariform larvae similar to those prepared by Melcher (1943). The fraction which gave the highest precipitating titer, 1 in 1,024, was polysaccharide in nature, but precipitating antibody was found at lower titers in all fractions. The possibility existed that antibodies against substances in the tissues of the worm were included with those that produced the oral and excretory precipitates. The tissue antibodies were absorbed from immune serum with an excess of various fractions extracted from the worms' bodies. Larvae then placed in the absorbed serum still demonstrated oral and excretory precipitates. Therefore,
it was concluded that the antibodies responsible for the formation of the precipitates were separate and distinct from those reacting with fractions from tissue extracts.

Oliver-González (1941, 1943) reported the successful absorption from sera of the antibodies responsible for the oral and excretory precipitates of *N. muris*.

Kagan and Bargai (1956), using agar diffusion tests, compared a saline extract of *T. spiralis* larvae with antigens fractionated by Melcher's (1943) methods. They found that his antigen was superior to saline extracts of *T. spiralis* larvae. It detected antibody earlier in infection and in one instance was positive when the saline antigen was negative. Agar double diffusion tests indicated that a minimum of three antigenic components were present in Melcher's antigen.

Oliver-González (1946) investigated the immunological relationships among polysaccharides from various helminths. Polysaccharide fractions were extracted from adult *A. lumbricoides, T. spiralis, Fasciola hepatica, Taenia saginata* and *Macracanthorhynchus hirudinaceus*. He observed a close immunological relationship between the polysaccharides isolated from *A. lumbricoides, T. spiralis, F. hepatica* and *T. saginata*. These helminths represent three large groups of parasites, nematodes, trematodes, and cestodes. The author concluded that cross-reactions may occur with all of these species of helminths during artificial immunization or infection.

Kagan (1957) employed the use of *A. lumbricoides, A. lumbricoides* var. *suum* and *Toxocara* sp. antigens in the diagnosis of visceral larva migrans. He prepared both whole worm and polysaccharide fraction antigens
from *A. lumbricoides* and *A. lumbricoides* var. *summ*. *Toxocara canis* and *T. cati* antigens were prepared from whole worms. Antisera were prepared, in rabbits, against *A. lumbricoides* var. *summ*, *A. lumbricoides*, *T. canis* and *T. cati* by injection of lyophilized antigen. Infection was produced by inoculation of the animals with viable eggs. The sera cross-reacted in hemagglutination tests. Absorption with heterologous antigen removed the cross-reacting antibodies and produced generic specific antisera. The sera obtained by injection of antigens showed higher titer than those obtained by infection produced with viable eggs. It was concluded that extracts prepared from pig ascarids are effective antigens in the hemagglutination test. Without preliminary absorption, antigens of *Toxocara* or *Ascaris* cross-react with antisera prepared against both species.

Kagan, Jesha and Gentzkow (1958) performed serum-agar double diffusion studies with antigens from various species of *Ascaris*. An antigenic analysis was done utilizing antigens made from *A. lumbricoides* var. *summ*. Polysaccharide-protein (prepared by a variety of methods), whole worm and autoclaved antigens were compared. Four technics were evaluated for the preparation of polysaccharide antigens. Ethanol precipitation, formamide followed by precipitation with acid alcohol and acetone, boiling in 30% KOH followed by ethanol precipitation, and precipitation with acetone of saline-soluble antigens. Polysaccharide-protein antigens from various *Ascaris* tissue fractions were also prepared. The antigen prepared with KOH showed only one band in the agar gel diffusion test against immune rabbit sera. The polysaccharide-protein antigens obtained with ethanol, formamide and acetone showed from four
to nine bands with the whole worm homogenate. Tissue antigens were less complex than whole worm antigens.

Of the five bands found in the whole worm polysaccharide-protein antigen, one component was isolated from the cuticle and a second immunologically distinct antigen from unembryonated eggs. All of the antigens tested contained protein and carbohydrate and were considered polysaccharide-protein complexes.

Jezioranski and Dobrowolska (1959) performed various serological tests for ascariasis using salt extracts and polysaccharide, nucleo-protein and lipoidal fractions of fresh or dried worms. All fractions were found to be active but none more sensitive than the whole worm extract of A. lumbricoides.
MATERIALS AND METHODS

Collection of larvae

The strain of *S. ratti* used in this investigation was obtained from naturally infected wild brown rats trapped in New Orleans, Louisiana, in 1949. The infection was established and maintained in white rats, two and one-half to three months old and approximately 200 gms. in weight. The animals were exposed to 1000 to 10,000 filariform larvae in five to seven ml. of distilled water, in a wide-mouth gallon jar covered with one-half inch wire mesh. The feet, legs and tails of the rats were in contact with the inoculum from 10 to 14 hours.

Filariform larvae were obtained by culturing fecal pellets from infected rats. Pellets were collected over a 24 hour period on wet paper toweling to prevent drying. They were placed in containers, mixed with animal charcoal and water and transferred to moistened paper toweling, cut to fit, in the bottom of petri dishes. The cultures were covered and kept at room temperature (25°C) for 24 hours. The dish tops were then moistened inside with a fine spray and allowed to remain an additional 24 to 48 hours at room temperature. At this time, the larvae were usually observed on the top of the petri dishes. They were collected by flooding inverted petri dish tops with water and removing the fluid with a pipette. The larvae were cleansed by two or three additional washings with distilled water. They were concentrated by centrifugation, quick-frozen and stored in a deep freeze until sufficient quantities were recovered for lyophilization. One ml. quantities of larvae were lyophilized in special glass bulbs on a Vir-Tis Freeze-Dryer. The bulbs were then heat-sealed under vacuum and stored in a deep freeze.

The strain of *S. fullerborni* used in this study was obtained from
naturally infected *Macacus rhesus* monkeys purchased from the Trefflich Co., New York, N. Y. The stools were collected, mixed with animal charcoal and treated in the same manner as previously described for the rat feces. The filariform larvae were collected, quick-frozen and lyophilized in the manner described for *S. ratti*.

The strain of *S. myopotami* was obtained from naturally infected nutria (*Myocastor coypus*) which were trapped by members of the Louisiana Wildlife and Fisheries Commission at the Rockefeller Wildlife Refuge, Cameron, Louisiana. The method of stool collection was slightly different for the nutria. The animal, being primarily a swamp inhabitant, requires a large container of water within its cage. Feces were collected from the container and larval cultures were made as done with the excreta of the rats and monkeys.

**Antigen preparation**

The method of polysaccharide extraction for the three species of *Strongyloides* was based on that of Melcher (1943). Lyophilized material was ground in a glass mechanical grinder and suspended in 20 volumes of 0.1N borate buffer, pH 8.0, placed in a boiling water bath and stirred for 45 minutes. The material was then cooled and centrifuged in a refrigerated (4°C) centrifuge for 15 minutes at 2000 rpm. The residue was washed once with borate buffer and this was added to the original extract supernatant. Precipitation of the polysaccharide and other soluble worm material was accomplished by the addition of 100 ml. of cold 95 percent alcohol. Precipitation was facilitated by the addition of sufficient sodium chloride to give approximately an 0.5 percent concentration. The solution was then held at 4°C for four hours. A
white gummy precipitate formed. This was removed by centrifugation and
resuspended in approximately 20 mls. of acetate buffer, pH 4.6. The
material which failed to dissolve was removed and discarded. The
solution was adjusted to pH 8.0 with 1.0M sodium carbonate solution.
The polysaccharide was again precipitated by the addition of sodium
chloride and cold 95 percent alcohol. This procedure of precipitation
at pH 8.0 and redissolving at pH 4.6 was repeated until the material
gave negative results when tested for protein by Biuret and Millon tests.
Eight to 10 precipitations were usually necessary to obtain this result.
The polysaccharide extract was then dried and stored in a deep freeze.
A positive Molisch reaction (for carbohydrate) was obtained on the
material. The polysaccharide was resuspended in distilled water for use
as antigen and stored in small volumes to eliminate frequent freezing
and thawing.

Agar gel diffusion tests

Agar gel diffusion tests were performed in tubes. The methods used
were similar to those of Oudin, J. (1946, 1948, 1952), and Ouchterlony,
O. (1949, 1950). In all tests, 0.5 percent Difco purified agar was
employed. In the simple diffusion tests 0.1 ml. of serum and 0.1 ml.
of 0.5 percent purified agar were mixed, placed in a tube and allowed
to harden. This was overlayed with 0.1 ml. of the antigen and the tube
sealed with plasticine. Results with this technic were difficult to
interpret and the method was discarded in favor of the double diffusion
tube method. In the double diffusion tube method, the inner surface of
the tube was first coated with a thin layer of agar. One tenth ml. of
serum and 0.1 ml. of agar were mixed, placed in the tube and allowed to
solidify. This was overlayed with 0.1 ml. of plain agar and allowed to harden. One tenth ml. of antigen was placed on top of the plain agar, the tube sealed with plasticine, and incubated at 37°C for varying time intervals. Fluorescent lighting against a black background was used for observing and photographing results. Doubtful band formations were checked by the use of a dissecting microscope.

**Paper chromatography**

Ascending paper chromatographic analyses of the polysaccharide fractions were performed. A 24" x 10" chromatocab was used for all separations. Sugars, chromatographically pure, from the Mann Research Laboratories, New York, N. Y., were used as reference solutions. The pentose and hexose sugars were used in 0.5 percent solutions of isopropanol and sugar alcohols and disaccharides in 0.7 percent solutions. Deoxyribose and rhamnose, which are comparatively insensitive to the locator reagents at these concentrations, required concentrations two or three times as strong. Numerous solvents—such as isopropanol and water; ethyl acetate, pyridine and water; n-butanol, pyridine and water; n-propanol, ethyl acetate and water; and isopropanol, n-butanol and water—were tested. The isopropanol (140 ml.), n-butanol (20 ml.) and water (40 ml.) solvent was chosen as it gave good separation and was less sensitive to temperature change. Whatman No. 4 Chromatography Paper was used for all separations. All antigens were desalted before being chromatographed to prevent tailing of the spots. This was accomplished by using a Torbal Chromatographic Desalting Apparatus, Model CD-1, utilizing ion exchange membranes and electric current.

Test solutions were spotted with a platinum loop two to three mm.
in diameter. This delivered approximately three microliters consistently. The loop could be easily and rapidly cleaned by insertion in a flame for a few seconds. The spots were confined to an area of 0.5 cm. by the use of a warm air blower while placing the loop of solution on the paper. Two locator reagents were employed. The first was a silver nitrate reagent consisting of two solutions: a 0.1 ml. of a saturated solution of silver nitrate in water, added to either 20 or 100 ml. of acetone; and a 0.5 percent solution of sodium hydroxide in 95 percent alcohol. The paper was first passed through the silver nitrate solution and the acetone evaporated with a warm air blower. It was then passed through the sodium hydroxide solution and the solvent evaporated again. Spots appeared at once and varied in color from brown to black. The second locator was an aniline-diphenylamine reagent which consisted of aniline, 1 percent, plus diphenylamine, 1 percent, in acetone and 85 percent phosphoric acid. After dipping the paper in this reagent it was heated to 95 to 100°C for a few minutes. Most sugars yielded green, blue or brown colors. Care was taken not to overheat the paper and cause its disintegration.

An attempt was made to find Rg values (Smith, Ivor, 1960) using glucose as a reference. These were determined by taking the distance the substance travels from the origin, dividing this by the distance glucose travels from the origin and multiplying the result by 100.

**Paper electrophoresis**

Paper electrophoresis was performed on the sera of patients and animals infected with the various species of *Strongyloides*. Electrophoretic serum patterns were run before and after absorption with an
excess of homologous and heterologous antigens. The patterns were run on a horizontal tank Spinco electrophoresis apparatus using a power pack calibrated from 0 to 500 volts. The buffer was prepared by dissolving 10.3 gms. of sodium barbitone in approximately 900 ml. of water and then adding 1.83 gms. of barbituric acid. Distilled water was added to bring the volume to one liter. All tests were performed using Whatman No. 1 paper. The current used was 100 volts for 16 hours. About 0.1 ml. of serum was used for each test run. The papers were removed and dried in an oven at 105 to 110°C. Drying was completed in approximately 10 minutes. The paper strips were stained with a one percent bromphenol blue in 95 percent alcohol saturated with mercuric chloride (30 gm./100 ml.). Plotting was done utilizing an Analytrol, recording, scanner and integrator, Spinco Model RB, with Spinco chart paper calibrated in centimeters.

Collection of blood samples

Blood samples were collected from patients at the Veterans Administration Hospital, New Orleans, Louisiana, who had a diagnosis of Strongyloides stercoralis proven by both stool examination and duodenal drainage. In some cases the samples were collected before, during and after treatment with dithiazanine. Rats were bled by heart puncture using ether as an anesthetic. Blood from the monkeys and nutria was collected by femoral vein puncture. The animals were exposed to 100 percent CO₂, in a cage draped with a plastic cover, as a knockdown anesthetic to facilitate the injection of sodium nembutal intraperitoneally for a longer period of anesthesia. All sera were stored in a deep freeze until used.
RESULTS

Agar gel diffusion tests

A polysaccharide antigen of _S. ratti_ filariform larvae was used against sera from rats infected with _S. ratti_ under various conditions. A single band appeared in 18 hours (fig. 1, pl. 1) in ten animals which had been exposed twice, at weekly intervals, to an inoculum of 5000 filariform larvae. A less distinct band appeared in 36 hours (fig. 2, pl. 1) in sera from ten rats which had been exposed to single inoculum of 2000 larvae. Bands did not form in 96 hours in ten animals with stool examinations negative for _S. ratti_ larvae and free of parasites at autopsy. Human sera with _S. ratti_ antigen gave similar results.

Of 60 blood samples drawn from patients infected with _S. stercoralis_, before, during and after dithiazone therapy, 57 showed a single band in 18 hours (fig. 3, pl. 1). The three samples which did not react were obtained while the patients were undergoing therapy. Five sera from monkeys infected with _S. fülleborni_ reacted showing one band in 48 hours. Eleven sera from nutria infected with _S. myopotami_ failed to react with the _S. ratti_ antigen. Examples of the types of reactions obtained at 18 and 48 hours using _S. ratti_ antigen with rat, monkey, human and nutria sera are shown in figure 4, plate 1, and figure 5, plate 2.

An antigen of _S. fülleborni_ filariform larvae was used with sera from five monkeys infected with _S. fülleborni_. Two bands appeared in 18 hours (fig. 6, pl. 2). One band appeared in 18 hours (fig. 7, pl. 2) in sera from patients with _S. stercoralis_ infections. An additional band appeared in 24 hours in 55 of the 60 serum samples (fig. 8, pl. 2).
The five samples which showed only one band were obtained during dithiazanine therapy. Twenty serum samples from rats infected with *S. ratti* showed no band formation. Of 11 sera from nutria infected with *S. myopotami*, two showed band formation; one a single weak band after 72 hours (fig. 9, pl. 3) and the other, two bands after 72 hours (fig. 10, pl. 3). The other nine did not react. An illustration of the type of reaction obtained at 18 hours using *S. fulleborni* antigen against sera from an infected monkey, human, rat and nutria is shown in figure 11, plate 3.

Antigen prepared from *S. myopotami* elicited little reaction. Five of the 11 sera from *S. myopotami* infected nutria showed one band after 72 hours (fig. 12, pl. 3). None of the five positive monkey samples reacted. The twenty rat sera did not show band formation. Fifteen of the 60 sera from patients infected with *S. stercoralis* showed a weak band in 72 hours. Nine of these samples were obtained before therapy, two during treatment and four after therapy. An illustration of the reactions observed at 72 hours using *S. myopotami* antigen against sera from an infected nutria, rat, monkey, and human is shown in figure 13, plate 4.

The three antigens—rat, monkey, and nutria—did not react with the sera from 20 patients infected with other helminths (*A. lumbricoides, T. trichiura* and hookworm) or with the sera from ten patients with negative stools and duodenal drainages.

Human sera drawn at intervals ranging up to two years after therapy, in the absence of demonstrable infection, still gave positive reactions with both *S. ratti* and *S. fulleborni* antigens (fig. 14, pl. 4).
Paper chromatography

Chromatographic studies of the polysaccharide antigens prepared from filariform larvae of S. ratti, S. fulleborni and S. myopotami revealed that the three antigens were different. S. ratti antigen moved more rapidly than the others. Its Rg value was 105 which is similar to fructose (104) under the conditions employed. The S. fulleborni antigen had a value of 64 which corresponds to glucosamine. The antigen prepared from S. myopotami had an Rg value of 62 which is between trehalose (60) and glucosamine (64). None of the three antigens was separated into more than one compound (fig. 15, pl. 5).

Electrophoresis

Serum electrophoresis patterns were obtained on human, monkey, rat, and nutria sera of infected individuals before and after absorption with homologous and heterologous antigens.

Human serum gamma globulin level before absorption was 3.1 cm. (fig. 16, pl. 6). After absorption with S. ratti antigen the level was 2.7 cm. (fig. 17, pl. 6), after S. fulleborni antigen 1.9 cm. (fig. 18, pl. 6), and after S. myopotami antigen 3.1 cm. (fig. 19, pl. 6).

The monkey serum gamma globulin level before absorption was 2.7 cm. (fig. 20, pl. 7). After absorption with S. fulleborni antigen, the level was 0.8 cm. (fig. 21, pl. 7), after S. ratti antigen 1.1 cm. (fig. 22, pl. 7), and after S. myopotami antigen 1.4 cm. (fig. 23, pl. 7).

The rat serum gamma globulin level before absorption was 1.5 cm. (fig. 24, pl. 8). After absorption with S. ratti antigen the level was 0.2 cm. (fig. 25, pl. 8), after S. fulleborni antigen 0.4 cm. (fig. 26, pl. 8), and after S. myopotami antigen 0.8 cm. (fig. 27, pl. 8).
The nutria serum gamma globulin level before absorption was 1.1 cm. (fig. 28, pl. 9). After absorption with *S. myopotami* antigen it was 0.9 cm. (fig. 29, pl. 9), after *S. ratti* antigen 1.0 cm. (fig. 30, pl. 9), and after *S. fülleborni* antigen 1.1 cm. (fig. 31, pl. 9).

In the serum pattern of a patient with numerous negative stool examinations and duodenal drainages, the gamma globulin level remained constant after absorption with each of the three antigens used (figs. 32, 33, 34, 35, pl. 10).
DISCUSSION

Pellegrino, Chaia and Memoria (1961) and Chaia (1962) used S. ratti filariform larvae as a whole worm antigen. These investigators used only stool examinations as a basis of determining infections in patients skin tested. The results of agar gel diffusion tests with a polysaccharide antigen performed in this study showed similar results to the skin tests except that the polysaccharide antigen showed no false positive reactions. In the present study, the absence of infection was better substantiated by stool examinations and duodenal drainages. In both studies the problem of duration of positive reactions was encountered. In the skin testing studies of Chaia (1962) positive reactions were obtained one year after therapy while in the present study positive precipitins were obtained as long as two years after therapy. In this investigation as in the studies by Chaia (1962) no cross reactions with other helminths occurred.

In gel-diffusion tests using the homologous system of S. ratti antigen and antiserum, a heavy band appeared in 18 hours. The heterologous system using S. stercoralis antiserum produced a light band in 18 hours, indicating a strong relationship. S. fulleborni antiserum produced a light band in 48 hours, indicating a weak relationship. S. myopotami antiserum did not react, and this would indicate no relationship under the conditions of this investigation. The homologous system gave the strongest reaction, and of the heterologous systems S. stercoralis antiserum was the most reactive. S. ratti antigen and S. myopotami antiserum did not produce a reaction. Both are rodent strains; however, S. myopotami filariform larvae will not infect white rats (Abadie, 1962).
Brannon and Faust (1949) used a species of *Stromgyloloeae* from the chimpanzee (thought to be *S. fulleborni*) to prepare whole worm antigens for skin testing and precipitins for the diagnosis of *S. stercoralis* infections. One hundred and eight patients determined to be non-infected, by stool examination only, were tested. Twenty-six gave positive tests ranging from doubtful to three-plus. In the present study the number of controls was smaller; however, the possibility of undetected infection with *S. stercoralis* was practically eliminated by stool examinations and numerous concurrent duodenal drainages. Eighteen of the 26 false positive tests encountered by Brannon and Faust (1949) involved patients with other helminth infections. The polysaccharide antigen used in this study produced no cross-reactions. The problem of antibody persistence following cure was again encountered using *S. fulleborni* antigen as with *S. ratti* antigen. The precipitin reaction with the *S. fulleborni* larvae polysaccharide antigen in human strongyloidiasis remained positive at least two years.

The homologous system of *S. fulleborni* antigen and antiserum showed two bands in 18 hours. This indicates two antigen-antibody systems. In the heterologous systems tested, *S. fulleborni* antigen with *S. stercoralis* antiserum was the most reactive, showing one band in 18 hours. The *S. ratti* antiserum did not react with the *S. fulleborni* antigen, indicating little if any relationship under the conditions of this investigation. A very weak relationship was shown between *S. fulleborni* and *S. myopotami* by the formation of faint bands in two of 11 antisera after 72 hours.

Antigens extracted from *S. myopotami* larvae showed the least activity of those tested. The homologous system showed only slight reactivity...
vity; five of 11 antisera produced only one band after 72 hours. In the heterologous systems only 15 of 60 antisera from patients with S. stercoralis had weak band formation after 72 hours. Antisera from both the rat and the monkey exhibited no reaction with the S. myopotami antigen. While the antigenic capacity of S. myopotami larvae was very slight, the antibody produced in the antisera was sufficient to elicit some slight reactions with the more potent antigens.

Campbell (1937) working with various helminths, other than Strongyloides sp., demonstrated that the specificity of precipitin reactions with polysaccharide antigen is far greater than with saline extracts of the whole worm. He found that polysaccharide fractions reacted only with homologous systems while the saline extracts also reacted with heterologous systems. In the present study, polysaccharide fractions reacted more strongly in homologous systems than in heterologous systems. However, there was a varying degree of activity in heterologous systems.

It can be concluded from the overall results of the agar gel diffusion tests that their use alone for the diagnosis of human strongyloidiasis is not feasible. The fact that antibodies persist for at least two years after termination of the infection makes this impractical. This test may be of value when used in conjunction with the patient's immediate symptomatology and the results of other laboratory tests.

Paper chromatographic studies revealed a different fraction from each of the three antigens. The three do not show any similarities from this study. In the present study, glucosamine was found in the S. filiemborni antigen but not in the other antigens. Williamson and
Desowitz (1961) reported the presence of glucosamine in the three flagellates, *Trypanosoma rhodesiense*, *Trypanosoma cruzi* and *Trypanosoma lewisi*. Chromatographic studies of the *Strongyloides* sp. antigens did not reveal species relationships like those shown by Nicks (1954) for mosquitoes. He used protein extracts and it is possible that similar fractions of *Strongyloides* sp. might reveal relationships.

Tiselius and Kabat (1939) showed that the removal of perceptible quantities of gamma globulin from serum by specific antibody could be demonstrated by means of electrophoresis. Results in the present study show that absorption in a homologous system removed more gamma globulin from the serum than did absorption in a heterologous system. This is demonstrated by a reduction in the height of the gamma globulin band. The patterns also show a close correlation with the results of the agar gel diffusion tests on similar systems. The quantity of gamma globulin in antisera from humans is most reduced (1.2 cm.) when absorbed with *S. fulleborni* antigen. *S. ratti* antigen produced less reduction (0.4 cm.), and *S. myopotami* antigen no reduction in gamma globulin. These results correlate with those of the precipitins which showed the strongest band formation between the patients' antisera and the *S. fulleborni* antigen. A lesser reaction was produced with the *S. ratti* antigen and a slight reaction with the *S. myopotami* antigen.

The greatest reduction in gamma globulin (1.9 cm.) was seen with the homologous system of *S. fulleborni* antigen and antiserum. The heterologous system of *S. ratti* antigen and *S. fulleborni* antiserum showed a reduction of 1.6 cm. while that of *S. myopotami* antigen and *S. fulleborni* antiserum only a 0.3 cm. reduction. This again correlates
with the precipitin studies as band formation occurred with S. ratti antigen and S. fulleborni antiserum.

This same relationship persisted when S. ratti antiserum was used. The largest reduction in gamma globulin (1.3 cm.) was seen in the homologous system and the next largest in the heterologous system using S. fulleborni antigen (1.1 cm.). The S. myopotami antigen displayed the least activity, reducing the gamma globulin to 0.7 cm. Once again a correlation with the precipitin reactions was demonstrated.

The S. myopotami antigen showed the least activity in the precipitin tests and also displayed the least activity in the absorption studies. The homologous system of S. myopotami antiserum and antigen reduced the gamma globulin content only slightly (0.2 cm.). In the heterologous system of S. myopotami antiserum and S. fulleborni antigen, it was reduced only 0.1 cm. and with S. ratti antigen no reduction was seen. These findings parallel those obtained with the precipitin test.

From the results of the several studies presented, the relationships between the various species may be determined. The various species, with the exception of the nutria strongyle, S. myopotami, appear closely related. S. stercoralis and S. fulleborni exhibited the closest relationship. There was some similarity between S. ratti and S. stercoralis. A relationship between S. ratti and S. fulleborni was demonstrated; however, both are more closely related to S. stercoralis than to each other. S. myopotami has the least relationship to the others, showing only a very slight relationship to S. fulleborni.
BIBLIOGRAPHY

Abadie, S. E. (Unpublished data, 1962.).

In parasitic infections in man. H. Most, editor. New York,

Brannon, M. J., and E. C. Faust. 1949. Preparation and testing of a
specific antigen for diagnosis of human strongyloidiasis. Am. J.

Campbell, D. H. 1937. The immunological specificity of a polysaccharide
fraction from some common parasitic helminths. J. Parasitol. 23:
4, 348-353.

Chaia, G. 1962. Contribuição Para O Estudo Da Reação Intradermica
Com Antígeno De Strongyloides ratti (Sandground, 1925) No
Diagnostico Da Estrongiloidose Humana Teste de Dantoramento
Apresentada A Escola De Farmacia de Duro Preto. Belo Horizonte.


Davies, D. A. 1955. The specific polysaccharides of some Gram-negative

Evans, E., and J. W. Mehl. 1951. A qualitative analysis of capsular
polysaccharides from Cryptococcus neoformans by filter paper

Exper. Path.u Pharmacol. 73:164-213.

Forsyth, W. G. C. 1948. Colour reagents for the paper chromatography


Schacher, J. F. 1956. Parasites of Louisiana mammals with emphasis on nutria (Myocastor coypus) and otter (Lutra canadensis, spp.). Final report to Louisiana Wildlife and Fisheries Commission.


CURRICULUM VITAE

Stanley Herbert Abadie. Born November 13, 1925; New Orleans, Louisiana.
Attended Holy Cross Elementary School, New Orleans, Louisiana.
1942 Graduated from Warren Easton High School, New Orleans, Louisiana.
1943 Entered U. S. Navy.
Served as Pharmacist's Mate in South Pacific until Honorably Discharged in June, 1946.
1949-55 Attended Loyola University, New Orleans, Louisiana.
Awarded B. S. Degree in August, 1955.
1956-58 Attended Louisiana State University Graduate School.
Awarded M. S. Degree in February, 1958.
1958-present Instructor in Department of Tropical Medicine and Medical Parasitology, L.S.U. Medical School.
EXAMINATION AND THESIS REPORT

Candidate: Stanley H. Abadie

Major Field: Medical Parasitology

Title of Thesis: The Immunology and Serology of Various Strongyloides Species

Approved:

[Signature]
Major Professor and Chairman

[Signature]
Dean of the Graduate School

EXAMINING COMMITTEE:

[Signature]

[Signature]

[Signature]

[Signature]

Date of Examination:

May 14, 1963