THE EFFECT OF PEPTIC AND TRYPTIC DIGESTION ON THE ANTIGENICITY OF *TRICHINELLA SPIRALIS*[1]

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(Received for publication August 5, 1946)

In the course of a previous investigation, positive trichinella skin tests which could not be accounted for by a history of known infection were encountered (1). An attempt was made to explain these findings by inducing reactivity in non-infected animals through repeated feedings of antigen (2). Killed larvae of *Trichinella spiralis,* presumably containing denatured (cooked) or undenatured (frozen) antigen, were fed to guinea pigs and rabbits. Attempts to demonstrate reactivity in these animals by skin tests and flocculation tests on the blood serum were unsuccessful. Because some investigators have found that digestion may alter the reactivity of pollens, it was thought that the negative results might have been due to digestion of the trichinella antigen (3, 4). The present study was undertaken to determine whether or not peptic or tryptic digestion would deactivate the antigen of killed trichinae.

MATERIALS

Animals

Fifteen young dogs weighing 5 to 15 kgm. were dewormed and divided into 3 groups of 5 each (groups A, B, and C). A fourth group of 15 dogs (group D) which had not been de-wormed was used as a control. All animals were fed a stock diet (Hunt Club Dog Food, Maritime Milling Co., Inc., Buffalo, N. Y.)

Trichinella larvae

The strain of *Trichinella spiralis* which we employed was obtained from the National Institute of Health and was carried in albino rats. Rabbits were given 1 feeding of trichinae-infected rat meat, and 8 weeks later were killed, skinned, and eviscerated. The muscle was removed, ground, and digested at 37° C. for 6 hours in a solution of 0.7 per cent pepsin and 1.0 per cent hydrochloric acid (5). The larvae were washed repeatedly in normal saline until the solution was biuret-negative, and were then frozen at −78° C. for 72 hours in carbon-dioxide ice, dried in a desiccator, and powdered.

[1] This study was aided by a grant from the John and Mary R. Markle Foundation.

METHODS

Preparation of the alkaline-trypsin digested antigen

Fifty mgm. of powdered trichinae were incubated for 48 hours with 5 ml. of 5 per cent trypsin (Armour and Co., Chicago, Ill.) in a 0.3 per cent sodium carbonate solution at 37° C. under anaerobic conditions. The anaerobic environment was chosen because Rockwell has suggested that oxidation may destroy antigenicity (6). As soon as the trichinae were added, a 22 × 200 mm. test tube was inverted over the 10 × 100 mm. unstoppered tube containing the digestion mixture, and a cotton plug was pushed into the larger tube to hold the smaller tube in place. The mouth of the larger tube was then placed in a beaker containing a mixture of pyrogallic acid powder and sodium hydroxide pellets. About 10 to 15 ml. of water were added to the reagents, and melted paraffin was poured on its surface. As the oxygen was utilized, the fluid column rose, effectively sealing the tube.

After digestion, the mixture was centrifuged for 30 minutes at approximately 3,000 RPM. The supernatant fluid was then removed with a pipet and neutralized to litmus with 5 per cent hydrochloric acid. Sufficient phenol was added as a preservative to make a final concentration of 0.04 per cent. A biuret test on the sediment was negative; the sediment was discarded.

A test for sterility on the solution of digested antigen revealed Gram-positive spore-bearing rods which were not further identified. Because of the contamination, the antigen was frozen for 48 hours in carbon-dioxide ice. A subsequent sterility test was negative. The Gram-positive bacilli were found to be a contaminant in the trypsin powder.

Preparation of the acid-pepsin digested antigen

Fifty mgm. of powdered trichinae were incubated for 48 hours with 5 ml. of 0.7 per cent pepsin (Merck, N.F. Granular) in 1 per cent hydrochloric acid at 37° C. under anaerobic conditions, by the method described above. The supernatant fluid, after centrifugation, was neutralized to litmus with 5 per cent sodium hydroxide. The sediment left after centrifugation was found to be very slightly biuret-positive; it was then discarded.

A sterility test on the digested antigen was negative, but in order to maintain conditions identical with the alkaline-trypsin digestion, the antigen was frozen for 48 hours in carbon-dioxide ice.

Preparation of the control antigen

Twenty-five mgm. of powdered trichinae were incubated anaerobically for 48 hours in 2.5 ml. of sterile 0.8

per cent sodium chloride solution. The mixture was then centrifuged and frozen exactly as in the preparation of the digested antigens. A biuret test on the sediment was strongly positive. A sterility test on the incubated antigen was negative.

Injections

Group A received the alkaline-trypsin digested antigen, group B the acid-pepsin digested antigen, and group C the undigested control antigen. Each animal was given an initial subcutaneous injection of 0.1 ml. of its respective antigen in the left pectoral region. One dog from each group (dogs 5, 10, and 14) was also given 0.1 ml. of its respective antigen intravenously on the same day, without reaction. Beginning 3 days later, all animals were given 3 successive daily intravenous injections of 0.1 ml. of their respective antigen, a total of 0.4 ml. of antigen to each dog, except dogs 5, 10, and 14, which received 0.5 ml.

Skin tests

In order to avoid the possibility of sensitization, no skin tests were performed before the injections were given. Nineteen days after the last intravenous injection, the abdomen of each animal in groups A, B, and C was clipped, and a skin test was done on each animal with a 1:100 dilution of antigen, prepared from desiccated and powdered trichinae recovered from the same rabbit that supplied the trichinae for the injected antigen. At the same time, each animal was given a skin test with a saline control. Subsequent skin tests with a 1:200 dilution of antigen from another rabbit were done at intervals of approximately 1 week through the sixth week after the last intravenous injection. All antigens used were found to give 4 plus reactions in a dog known to be infected with trichinae.

The reactions were read at 20 minutes. A 1 cm. wheal with surrounding erythema was considered to be a 1 plus reaction, if the skin test with a saline control produced a wheal less than 8 mm. in diameter. Reactions were graded progressively from 1 plus to 4 plus.

As a further control against non-specific or group skin reactions, 15 additional dogs (group D) which had not been de-wormed, or given any injections of antigen, were given skin tests with a 1:200 dilution of antigen.

Flocculation tests

Preliminary flocculation tests were done on the serum obtained from each dog in groups A, B, and C 9 days before the injections of antigen were begun. Eleven days after completion of the intravenous injections, 5 ml. of blood were obtained from the antecubital vein of each dog. The serum was separated and inactivated at 56° C. for 30 minutes. Tests were performed according to the technique of Suessenguth and Kline (7), except that the rotation was continued for at least 10 minutes. Judging by the negative control, this longer time interval did not give false positive results. Minimal but definite flocculation which could be seen only under the microscope was considered a doubtful reaction (±). Flocculation tests were repeated at intervals of approximately 1 week through the sixth week after the last intravenous injection.

RESULTS

Skin tests

In 13 of the 15 dogs in groups A, B, and C, skin tests were found to be positive on the nineteenth day after the initial injection, and continued to be positive for the duration of the experiment (Table I). One animal (dog 5) in group A (alkaline-trypsin digested antigen) failed to develop a definitely positive skin test during the 6-week period of observation. One animal (dog 9) in group B (acid-pepsin digested antigen) failed to react at 19 days, but subsequently developed a positive skin test. No difference in the extent of reactions was noted among the 3 test groups.

TABLE I

Skin tests

	Dog	Days following initial injection			
		19	24	30	41
Group A Alkaline-trypsin digested antigen	1	++	+	+	++++
	2	+++	++++	++++	++
	3	+	++++	++++	++++
	4	++	++	+	++++
	5	0	0	±	±
Group B Acid-pepsin digested antigen	6	+	+++	++	++
	7	+	++	++++	++++
	8	+	0	±	++
	9	0	+	+	+
	10	+	±	+	++++
Group C Saline antigen control	11	+++	++	++	++
	12	+	+++	+++	++
	13	+	+++	++	++++
	14	+	++	++	++++
	15	++	++++	++++	++++

Of the 15 dogs in group D (the control group of dogs which had not been de-wormed and had not received injections of antigen), 5 gave negative reactions, 5 gave doubtful (±) reactions, 4 gave 1 plus reactions, and 1 gave a 2 plus reaction at 20 minutes. Two gave 1 plus reactions at 24 hours.

Flocculation tests

Preliminary flocculation tests on the sera of all dogs in groups A, B, and C were negative (Table II). On the twenty-first day, tests on all these dogs gave at least doubtful reactions. No striking difference in the flocculation tests was noted among

TABLE II

Flocculation tests on serum

	Dog	Pre-liminary	Days following initial injection			
			16	21	35	41
Group A Alkaline-trypsin digested antigen	1	0	0	++	±	+
	2	0	0	+	±	+
	3	0	0	±	±	+
	4	0	0	±	0	±
	5	0	0	+	±	+
Group B Acid-pepsin digested antigen	6	0	0	+	++	++
	7	0	0	++	0	0
	8	0	0	+	±	0
	9	0	0	++	+	+
	10	0	0	+++	+	+
Group C Saline control antigen	11	0	0	+	±	±
	12	0	0	±	±	0
	13	0	0	+	±	±
	14	0	0	++++	±	±
	15	0	0	+++	+	±

the 3 test groups. In general, the skin tests gave more strongly positive reactions than did the flocculation tests.

DISCUSSION

The effect of digestion on antigenicity

Acid-pepsin and alkaline-trypsin digestion of trichinella antigen did not destroy its reactivity for dogs, as measured by skin tests and flocculation tests on serum. Our results suggest that the antigenicity of trichinae does not reside solely in the protein fraction. Jadassohn found that peptic digestion of ascaris antigen did not alter its reactivity for skin tests (8). The results of our studies on another helminth parasite agree with his findings.

The reports in the literature concerning the effect of digestion on the antigenicity of grass pollens are conflicting. Coca and Grove were unable to find any diminution, as measured by skin tests, in the activity of ragweed and timothy pollen extracts after digestion with trypsin (10). Black obtained similar quantitative reactions with ragweed pollen extracts, as measured by endermic and intranasal tests, before and after tryptic digestion (11). He concluded that the substance responsible for pollen sensitization is not a protein, that it survives passage through the alimentary tract, and that it can be recovered from blood, urine, and feces (12). Thiberge, however, showed a reduction in the skin-test activity of ragweed pollen extracts after artificial digestion with pepsin and hydrochloric acid (3). Pancreatin digestion was said to intensify the reactivity of these extracts, but details of the technique used were not given (9). Harsh and Huber found that peptic or tryptic digestion of giant ragweed pollen caused a marked loss of activity of the antigen, as measured by scratch and intracutaneous tests (4). A small but constant amount of activity which was unaffected by proteolytic digestion remained.

Thiberge demonstrated by a passive transfer test (Prausnitz-Kustner phenomenon) that ragweed antigen was absorbed in an active form from the intestinal tract after being administered in enteric-coated pills. The preponderance of evidence indicates that the proteolytic enzymes of the gastro-intestinal tract destroy little of the active substance in pollen antigen. Little is known concerning the effect of carbohydrate-splitting enzymes on the antigen.

The experiments reported here do not explain, on the basis of digestion of antigen, the failure to induce reactivity to trichinella antigen in animals by repeated feeding of killed trichinae (2). The possibility that ingestion of killed trichinae by human beings may cause sensitization without actual infection still remains (1).

The antigenicity of trichinae fractions

Melcher, by chemical methods which did not employ enzymatic digestion, separated a polysaccharide and 2 protein fractions from trichinae (13). In infected rabbits, the protein fractions gave positive skin reactions at 24 hours, while the polysaccharide gave negative skin tests at 24 hours, but gave strongly positive precipitin reactions. The polysaccharide also induced precipitin formation when injected in non-infected animals, and the serologic studies suggested that the carbohydrate was associated with the acid-soluble protein fraction. Our experiments with dogs would suggest that the positive skin reactions at 20 minutes to antigen freed of protein by digestion might be due to a carbohydrate which need not be the same as that studied by Melcher. The experiments were done on a different species of animal, and the tests were read at a different time, but in human beings with pneumococcal infections the reaction in the skin to injected polysaccharide occurs at 20 minutes.

Bachman reported that the active antigenic fraction of trichinae is a glucoprotein, a substance chemically similar to mucin and containing carbohydrate in the molecule. He did not attempt to split the compound and study the protein and carbohydrate fractions separately (14). Cross-reactions between trichinae and other parasites, which occur at low dilutions of antigen (1:100), tend to disappear at high dilutions (1:10,000) (15). It is not known whether the cross-reactions are associated with a polysaccharide or with a protein fraction.

Proteolytic digestion of the trichinella antigen employed for skin tests may be found to reduce the incidence of non-specific false positive reactions commonly encountered with the protein-containing antigen now used.

The relative sensitivity of skin test and serologic methods

The fact that the skin tests gave more strongly positive reactions than the flocculation tests may indicate that the skin gives a greater quantitative response, and hence that the skin test is more sensitive. However, dogs known to be infected give 4 plus reactions to both skin and flocculation tests. The difference in intensity of reaction between the flocculation tests and the skin tests might be explained on the basis of 2 antigens, one elaborated continuously by the living larvae and resulting in flocculating antibodies; the other, an incomplete antigen incapable by itself of producing flocculating antibodies, and requiring the presence of another antigen not found in extracted whole killed trichinae, but present in living trichinae. Recent experiments by Roth suggest that precipitation of low titer immune serum around *living trichinae* is a more sensitive test than precipitation produced by antigen from dead parasites (16).

SUMMARY

1. Acid-pepsin and alkaline-trypsin digestion of *Trichinella spiralis* antigen did not destroy its antigenicity for dogs, as measured by skin tests and flocculation tests on serum.

2. The more marked reactivity of the skin test as compared with the flocculation test suggests that the same antigen may not be responsible for both reactions.

3. The failure of proteolytic digestion to destroy the antigens suggests that the antigenic fraction of *Trichinella spiralis* is not solely a protein.

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