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ANTIGENIC PROPERTIES OF PURIFIED FRACTIONS FROM ASCARIS LUMBRICOIDES VAR. SUUM ON NATURALLY SENSITIZED GUINEA-PIG

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Purified extract of *Ascaris* injected intravenously into guinea-pigs produced a reaction very similar to the anaphylactic reaction. As shown by the ratio of deaths to survivals the toxic fraction contained in *Ascaris* extract was purified 3 to 4 times. After intravenous injection animals presented both anaphylaxis-like shock and mast cell damage similar to that observed in anaphylaxis. Perfusion of the lungs with *Ascaris* fractions released histamine. Isolated guinea-pig ileum when in contact with *Ascaris* fractions showed a response followed by desensitization. Heating the ileum to 45° C or previous desensitization to anaphylatoxin excluded the participation of the latter in this contraction. *In vitro*, sensitization from one guinea-pig could be transferred to another, using spleen extract or a 50% ammonium sulphate precipitate obtained from the spleen extract. On the basis of these results it is concluded that guinea-pigs are naturally sensitized to antigen or antigens present in *Ascaris lumbricoides*.

Shinamura & Fuji (1917) isolated from *Ascaris lumbricoides* an "albumose-peptone" which they designated as "askaron," a very toxic substance for dogs. In 1939 Machebouef & Mandoul showed that deproteinized extract from *Ascaris megalocephala*, when injected intravenously into guinea-pigs, produced a reaction reminiscent of anaphylaxis. Bier (1939) showed that *Ascaris lumbricoides* contained a substance very active in producing an anaphylaxis-like shock in guinea-pig. Rocha e Silva & Grãna (1946), using a deproteinized and dialysed extract from *Ascaris lumbricoides*, were able to produce fatal shock in dogs and guinea-pigs. Further purification of this material led them to identify the active fraction as a proteose of high molecular weight. Nakajima (1954), after heating to 80° C an aqueous extract of *Ascaris lumbricoides*, purified it by alcohol precipitation, and obtained an active fraction containing nitrogen and phosphorus. Högberg, Thufvesson & Uvnäs (1956), precipitating *Ascaris* extract in alcohol followed by passage through an Amberlite IRC-50 XE-64 column, obtained an active fraction which released histamine from perfused paw of the cat. Recently, Gazzinelli, Guia, Neves, Pudles, Beraldo & Dias da Silva (1961) showed that the active fraction of the *Ascaris lumbricoides* was a complex substance containing carbohydrate and protein.

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Furthermore, they suggested that the guinea-pig is naturally sensitized to *Ascaris lumbricoides*.

In the present paper the chemical analysis of the active fraction was extended and its further purification explored. In addition the mechanism of action of this fraction on the guinea-pig was studied.

METHODS

Guinea-pigs of either sex, body weight 200 to 400 g, were used without any previous sensitization.

Intravenous injection into unanaesthetized guinea-pig. The injections were made using the veins of the front legs by means of a 1 ml. tuberculin syringe. The volume of the solutions injected did not exceed 1 ml.

Mast cell observation. The lungs were fixed as described by Mota & Vugman (1956). The mast cell number was assessed by counting thirty fields, at a magnification of 900, and the reduction in the number of the cells was expressed as % of the control.

Perfusion of the lungs. The animals were anaesthetized with ether. The trachea was cannulated and the thorax opened. A polythene cannula was inserted into the pulmonary artery and the perfusion started. Another cannula was tied into the left ventricle for the collection of perfusates. The preparation (lungs, thorax and head) was kept at about 38° C. The lungs were perfused with Tyrode solution at 38° C, at a rate ranging between 1 and 1.5 ml./min. After washing the lungs free of blood as completely as possible two 5 ml. samples were collected as controls. The perfusion was stopped and 0.5 to 1 ml. of a solution, containing *Ascaris* fraction, was injected through the cannula inserted into the pulmonary artery, by means of a tuberculin syringe. The injected solution was allowed to remain in contact with the pulmonary tissues. Five min later the perfusion was continued until only traces of histamine could be detected in the perfusates.

Histamine assays were performed on the atropinized guinea-pig ileum according to Feldberg & Talesnik (1953). All histamine values are given as base.

Purification of active fractions from Ascaris lumbricoides. *Ascaris lumbricoides* collected from hogs were treated according to the method described by Rocha e Silva & Grãna (1946). 875 g of *Ascaris* yielded 850 mg of an active fraction, called SB_1; 765 mg of this material was dissolved in 60 ml. 1 M acetate buffer of *p*H 5.2 at 4° C; ammonium sulphate was added until a flocculent precipitate began to form. The precipitate was centrifuged at 3,000 r.p.m. at 4° C, the supernatant collected and dialysed against distilled water for 24 hr in the cold room and then lyophilized. 405 mg of the lyophilized material was dissolved in 6 ml. 0.01 M phosphate buffer of *p*H 7.5 and was run through a column (1 × 55 cm) of hydroxyapatite. When the same buffer was used for elution and 3 ml. eluates were collected, the active substance appeared in the eluates nos. 18 to 30. These eluates also showed the highest absorption for protein at 280 mμ. Further elution with higher ionic strengths gave other protein peaks which still contained some activity. The yield of active eluates nos. 18 to 30, after 48 hr dialysis against distilled water and lyophilization, was of 69 mg (fraction SB_3). The rechromatography of the fraction SB_3, on a column of Amberlite IRC-50, XE-64, showed only one protein peak. Total nitrogen was estimated by micro-Kjeldahl and Nesslerization. For protein determination the method of Lowry (1951) was employed using serum as standard. The ultraviolet absorption method of Warburg and Christian (1941) was used for comparison. Organic and inorganic phosphorus were determined according to Nakamura (1952), the reducing sugars by the method of Somogyi (1945). Each fraction was hydrolysed in a sealed tube with 3 N hydrochloric acid for 5 hr in a boiling water bath. After neutralization, an aliquot was taken and estimated. The results were expressed in terms of glucose. The amino sugars were estimated by the method of Elson and Morgan (1933) and the results expressed in terms of glucosamine. The analytical data presented in Table 1 show the maximum and minimum amounts of proteins, reducing sugars and nitrogen obtained from seven different *Ascaris* batches.

TABLE 1

CHEMICAL ANALYSIS OF FRACTIONS SB_1 AND SB_3 FROM *ASCARIS LUMBRICOIDES*

Fraction	Protein (%) Lowry	Protein (%) 280/260 mμ/mμ	Reducing sugars (%)	Amino sugars (%)	Nitrogen (%)	Total phosphorus (%)
SB_1	27·4–29	33	15–23·3	6·6	7·7–8·7	1·18
SB_3	7·6–11	7·7–10	35–42	14	3·4–5·5	1·80

The analytical data of amino sugars and total phosphorus refer only to the preparations containing the minimum amount of nitrogen, protein and reducing sugars. In all purified fractions a positive Sakaguchi, a feeble Molisch and negative Ehrlich reaction for sialic acid were found (Blix, 1936). The test for free sulphydryl groups was also negative.

Substances used. Histamine diphosphate (Nutritional Biochemicals Corporation). Diphenhydramine hydrochloride (Benadryl, Parke, Davis). Anaphylatoxin was prepared according to Rocha e Silva & Aronson (1952).

RESULTS

Intravenous injection of Ascaris *fractions.* The majority of the animals, when injected with purified fractions of *Ascaris*, showed a reaction very similar to anaphylactic shock. The activity of the fractions SB_1 and SB_3 was determined by recording the ratio of deaths to survivals, in groups of 6 animals, as shown in Table 2.

TABLE 2

RELATION OF DOSE (μG/100 G) TO MORTALITY FOR GUINEA-PIGS INJECTED WITH SB_1 AND SB_3 FRACTIONS

Ascaris fraction	Total weight (μg)	Protein (μg)	Residue (μg)	Mortality ratio
SB_1	50	15	35	0/6
	133	40	93	3/6
	200	60	140	5/6
SB_3	132	10	122	4/6
	264	20	244	5/6

TABLE 3

REDUCTION IN MAST CELL NUMBER IN GUINEA-PIG LUNG AFTER INTRAVENOUS INJECTION OF 30 μG/100 G *ASCARIS* FRACTION SB_3

Mast cell numbers are the means of thirty microscopical fields at a magnification of ×900

Guinea-pig	Injected with	Number of mast cells per field	(means)	% of reduction
1	1 ml. sodium chloride 0·9%	14		
2		10		
3		12	14	—
4		18		
5		16		
6	SB_3 in 1 ml. sodium chloride 0·9%	2		
7		1		
8		5	3	78·5
9		3		
10		4		

Mast cell damage. Microscopic examination of the lungs of the guinea-pigs receiving intravenous injection of *Ascaris* fractions which died in shock showed constant and well-defined alterations in the mast cells. These were characterized by disappearance of granules (degranulation) and a consequent reduction in the number of stainable mast cells. As may be seen in Table 3, the *Ascaris* fractions produced a 78.5% reduction of the number of the mast cells.

Histamine release from the perfused guinea-pig lungs. In 6 experiments, perfusion of the isolated guinea-pig lungs with purified fractions from *Ascaris lumbricoides* was accompanied by the release of histamine 0.9 to 5.8 μg. Fig. 1 shows the output of histamine following the injection of SB_3 fraction. Ten min after the injection, 1.5 μg (46%) of the liberated histamine appeared in the perfusate and 40 min after the injection only traces of histamine were found in the perfusate. As a rule, the second injection of same amount or even of greater amounts of the *Ascaris* fraction yielded only about one-half of the total histamine released by the first injection.

Experiments with the isolated guinea-pig ileum. Guinea-pig ileum fragments 2.5 to 3 cm long were suspended in 10 ml. atropinized Tyrode solution. The addition of 1 to 10 μg of the *Ascaris* fractions to the bath containing the guinea-pig ileum

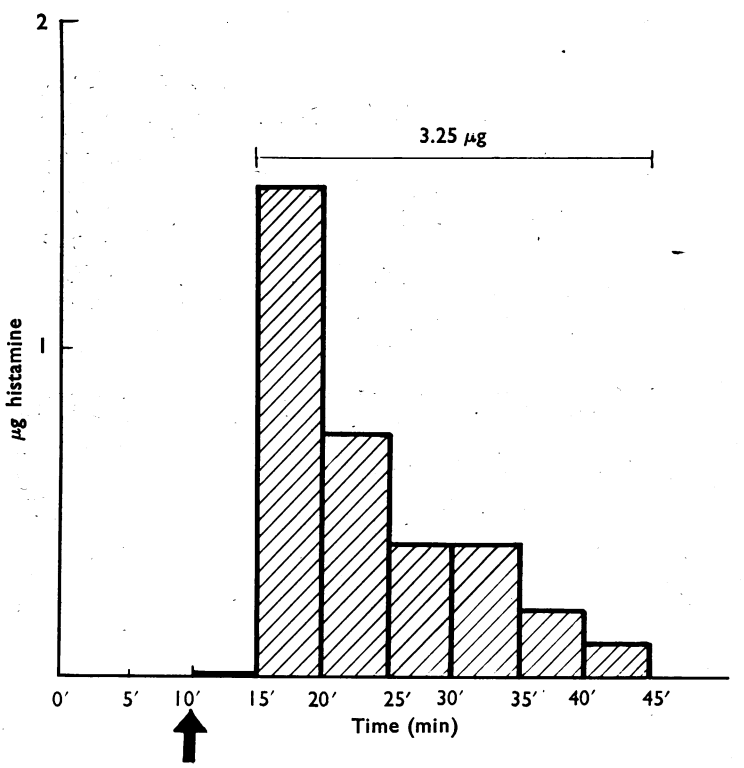

Fig. 1. Histamine release from perfused guinea-pig lungs. The arrow indicates the injection of SB_3 fraction 28 μg into the cannula.

evoked, after a period of latency about 60 to 90 sec, a strong contraction. A second addition of the same amount was either ineffective or produced a small effect, followed by a refractory state (desensitization) to a further addition of the *Ascaris* fractions. The response of the ileum and its desensitization was observed in 30 out of 35 different preparations tested.

In order to verify whether the desensitization of the ileum would occur also *in vivo*, 7 guinea-pigs were injected intravenously with sublethal doses of *Ascaris* fractions. Two days later the ilea were suspended in an organ bath and challenged as described above.

None of the ilea of the desensitized guinea-pigs responded when *Ascaris* fraction was added to the perfusion bath.

Possible participation of anaphylatoxin. Mongar & Schild (1957) showed that previous heating at 45° C of guinea-pig tissues inhibited the anaphylactic reaction *in vitro*. When anaphylatoxin was used, however, this effect was not observed (Mota, 1959).

In order to investigate whether the contraction of the guinea-pig ileum produced by *Ascaris* fractions was due to an anaphylatoxin-like substance present in the *Ascaris*, it was decided to try the effect of heat on this reaction. To test this hypothesis two fragments of the same ileum were taken. The first one (control) was

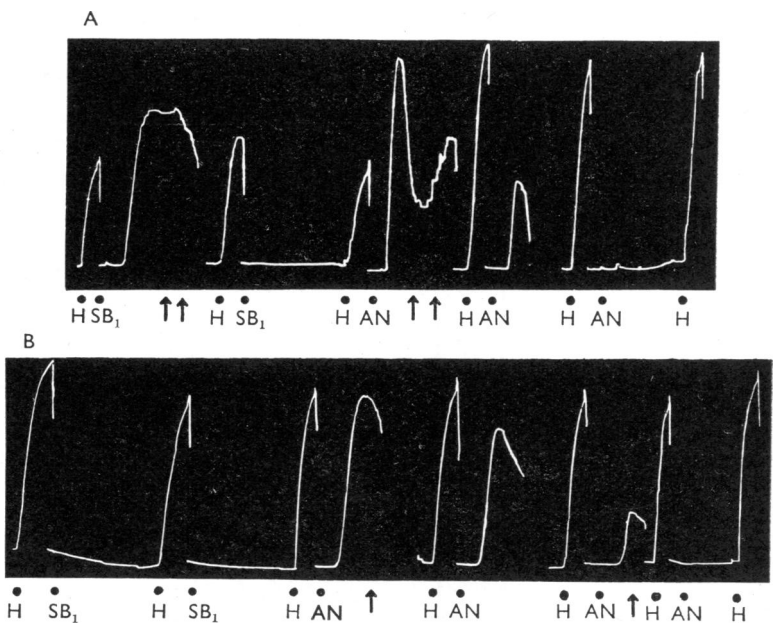

Fig. 2. Guinea-pig ileum preparation suspended in 10 ml. Tyrode solution. *Tracing A*, H=0.1 μg histamine; SB_1=100 μg *Ascaris* fraction; AN=0.1 ml. anaphylatoxin. *Tracing B*, The ileum was previously heated at 45° C for 15 min; H=0.1 μg histamine; SB_1=100 μg *Ascaris* fraction. AN=0.1 ml. anaphylatoxin. The arrows indicate washings with Tyrode solution. The substances were added to the bath at 2 min intervals.

desensitized to *Ascaris* fraction (SB_1). Even in this condition, the preparation produced a very strong contraction when anaphylatoxin was added to the bath (Fig. 2, A). The second fragment of the ileum was heated at 45° C, for 15 min, suspended in Tyrode solution at 35° C, and the effect of *Ascaris* fraction (SB_1) and anaphylatoxin tested. The heated fragment gave no response to *Ascaris* fraction, but gave a strong response to anaphylatoxin (Fig. 2, B). Besides, previous desensitization of ileum to anaphylatoxin does not affect the response to *Ascaris* fraction. As was observed by Mota (1959), in a guinea-pig ileum desensitized to anaphylatoxin, a subsequent addition of the specific antigen still produces the anaphylactic response.

Passive sensitization in vitro. As the participation of anaphylatoxin in the reaction produced by *Ascaris* fractions was excluded, the possibility was examined that an antigen-antibody reaction was involved in this contraction. To test this, fragments of guinea-pig ileum, previously made refractory to *Ascaris* fractions, were incubated at 35° C for 2 hr with serum collected from a guinea-pig whose ileum was tested and found to be reactive to the *Ascaris* fractions. After incubation, the ileum was challenged with *Ascaris* fraction and it was observed that in 2 out of 6 experiments the sensitivity had been transferred from the guinea-pig serum to the ilea. In an attempt to increase the frequency of the passive sensitization, guinea-pig spleen extract was tested in view of experiments by several authors showing that spleen is an organ which produces and stores antibodies (Stavitsky, 1955 ; McKenna & Stevens, 1957). Spleen extracts were prepared in a glass homogenizer and distilled water was added in order to make a final concentration of 10%. After centrifugation the supernatant was collected ; sodium chloride and glucose were added in the same proportion as in the Tyrode solution. This extract was used for passive sensitization as follows: guinea-pig ilea previously desensitized to *Ascaris* fraction were incubated with the supernatant for 2 hr at 35° C. They were then washed with Tyrode solution and challenged by adding *Ascaris* fractions to the bath. In 8 out of 12 experiments the ileum contracted when in contact with *Ascaris* fraction. A second or third addition of *Ascaris* fraction caused only a small response of the ileum or no response at all. Furthermore, the precipitate obtained from spleen extract by ammonium sulphate retained the ability of transferring passive sensitization, as can be seen in the following experiment: 20 ml. spleen extract was precipitated by adding ammonium sulphate to 50% saturation. The precipitate was dissolved in 10 ml. distilled water and dialysed against tap water for 48 hr at room temperature and the dialysis was continued against saline at about 4° C for 10 to 12 hr. This material was used for the *in vitro* passive sensitization. Fig. 3 illustrates one of these experiments.

In order to verify the specificity of the passive sensitization spleen extracts were prepared from guinea-pigs with ilea insensitive to *Ascaris* fractions (negative Schultz-Dale-like reaction). In 5 experiments in which different spleen extracts were used sensitization could not be transferred to the ileum. In addition, the specificity of the *Ascaris* fractions to produce contractions of the passively sensitized ileum was also investigated using other antigens. Fig. 4 shows that no contraction of the ileum was obtained when horse serum or ovalbumin was used, although the ileum contracted when *Ascaris* fractions were added to the bath.

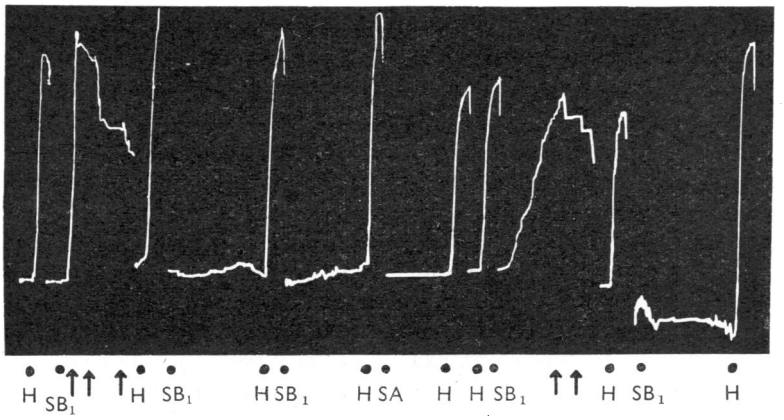

Fig. 3. Guinea-pig ileum suspended in 10 ml. Tyrode solution: H=0.05 μg histamine. SB_1=130 μg *Ascaris* fraction. SA=10 ml. spleen extract precipitate (50% ammonium sulphate saturation) dissolved in distilled water and dialysed against saline (see text), allowed to remain in the bath for 2 hr. The arrows indicate washings with Tyrode solution. The substances were added to the bath at 2 min intervals.

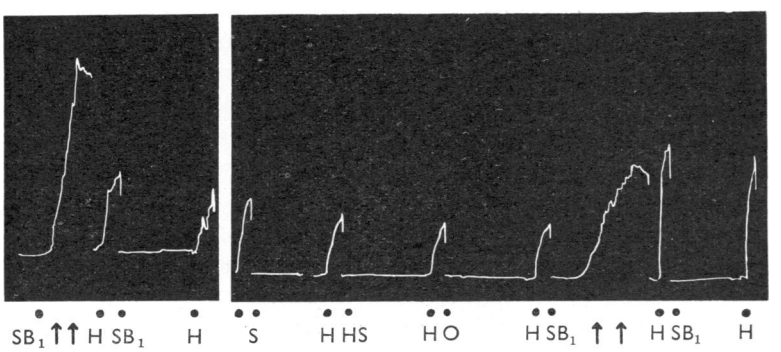

Fig. 4. Guinea-pig ileum preparation in 10 ml. Tyrode solution: SB_1=130 μg *Ascaris* fraction H=0.1 μg histamine. S=10 ml., 10% spleen extract, allowed to remain in the bath for 2 hr. HS=0.1 ml. horse serum. O=1 mg ovalbumin. The arrows indicate washings with Tyrode solution. The substances were added to the bath at 2 min intervals.

DISCUSSION

The intravenous injection of *Ascaris* extract produced in the guinea-pig a fatal anaphylaxis-like reaction. These experiments confirm those described by Bier (1939) and Rocha e Silva & Grãna (1946).

The perfusion of the guinea-pig lungs with purified *Ascaris* fractions led to a release of histamine which ranged between 0.9 and 5.8 μg. These results agree with those obtained by Bartosch, Feldberg & Nagel (1932) for guinea-pig lungs perfused with specific antigen. In addition, the alterations of mast cells produced by purified

Ascaris fractions were very similar to those induced by anaphylatoxin (Mota, 1959) or specific antigen in sensitized guinea-pig (Mota & Vugman, 1956).

The experiments with isolated ileum showed that *Ascaris* fractions are able to produce a strong contraction of this organ, indistinguishable from that induced in anaphylaxis (Schultz, 1910a, b ; Dale, 1913) or by anaphylatoxin (Rothschild & Rocha e Silva, 1954). However, the results with the ileum heated to 45° C or previously desensitized to anaphylatoxin excluded the participation of the latter, and suggested an antigen-antibody reaction in the mechanism of this contraction (Mongar & Schild, 1957 ; Mota, 1959).

This problem was further studied by sensitization of ilea by spleen extract *in vitro*. It was also observed that a 50% ammonium sulphate precipitate obtained from spleen extract retains the ability of sensitizing the ileum. The possibility of spontaneous or non-specific resensitization, in the absence of sensitive spleen extract, was excluded by experiments on spleen extracts from guinea-pigs with ilea insensitive to the *Ascaris* fractions. Our experiments therefore support the view that a passive sensitization by antibodies present in spleen extract was obtained. These results do not mean that spleen extract is richer in antibodies than serum, for in these experiments no comparative study between serum and spleen extract was performed.

Those guinea-pigs in which the intravenous injections of the *Ascaris* fractions did not cause any effect also showed no mast cell damage. In such guinea-pigs the ilea proved insensitive to the *Ascaris* fractions and the release of histamine from the lungs did not occur ; furthermore, the spleen extracts obtained from these animals were unable to transfer the passive sensitization.

These findings suggest that all effects produced by *Ascaris* extracts on the guinea-pig depend on a natural sensitization of the animals to antigen or antigens present in the *Ascaris lumbricoides*.

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