

ASCARIS SENSITIZATION¹

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It is a well established fact that *Ascaris lumbricoides*, the common intestinal roundworm of human beings and pigs, and other more or less closely related species parasitic in various animals, have toxic properties. This is clear not only from the nature of the symptoms in many cases of infestation which can be explained only on the basis of a toxic origin but also from the experience of many persons, mostly laboratory workers, who have had occasion to handle these parasites or who have in some way been brought into contact with them. Zoologists, for example, who have made extensive use of the horse *Ascaris* in cytological studies, have been frequent sufferers from its poisonous effects; Goldschmidt (1910) who was himself susceptible discovered by inquiry among zoologists of his acquaintance about 20 cases of similar susceptibility. (2).² Prior to Goldschmidt various writers, Bastian, Huber, Railliet, Linstow, and others, had reported personal experiences with the toxic effects of *Ascaris*, involving either the horse *Ascaris* or the human *Ascaris*. In fact susceptibility to *Ascaris* toxins is a very common condition among biologists who have been much exposed to contact with the parasite. Thus *Ascaris* sensitization is a sort of occupational disease. Its significance in relation to the toxic action of *Ascaris* in cases of infestation with the parasite remains to be determined.

The symptoms in persons who are sensitive to contact with *Ascaris* are of a kind that immediately suggest a similarity to the symptoms seen in cases of hay fever, asthma and other conditions grouped under the heading of foreign protein sensitization. Among the symptoms are irritation of the mucous membranes of the eyes, nose, and throat, lachrimation and edema of the eyes, facial edema, sneezing, coughing, swelling of the nasal mucosa, increased nasal and bronchial secretions, painful deglutition, urticaria, asthma, headache, fever, pruritus, tingling and burning sensations, swelling of the fingers, lassitude and weakness, sometimes amounting to prostration. In some cases the asthmatic attacks have persisted for weeks after exposure, but it is not certain in these cases that the possibility of subsequent exposures was entirely excluded. It has been commonly stated that the offending substance or substances are given off as emanations by the worm and thus are presumably volatile, but that they are actually volatile in the usual sense of the word has not been finally proved, although Weinberg and Julien (8) obtained a positive ophthalmic reaction in one out of 25 horses tested with the products of distillation of the body-cavity fluid of the horse ascarid, a result tending to support the prevalent belief in the presence of volatile substances in *Ascaris* that will produce symptoms in sensitive persons. Our own investigations thus far have failed to demonstrate a volatile substance that causes symptoms in human subjects sensitive to *Ascaris*, but we have not yet in our work taken up the question of those fractions of the worm that are insoluble in water.

¹ Received for publication May 12, 1924.

² Reference is made by number (*italic*) to "Literature cited," p. 582.

The horse *Ascaris* appears to be more powerful in its effects than the human *Ascaris*, judging from the fact that more severe symptoms have been commonly reported among persons sensitive to the former than among those sensitive to the latter, but this may be related to the fact that the horse *Ascaris* has been in general more intensively worked with by zoologists than the human *Ascaris*, so that exposure has commonly been greater in the case of the former. Individuals may be sensitive to both species, as in the case of Goldschmidt, or sensitive to one and not to the other, as in the case of Bastian, in whom the horse *Ascaris* would cause severe asthmatic attacks but upon whom the human *Ascaris* had no effect. Whether an individual may be sensitive to the human *Ascaris* and not to the horse *Ascaris* has not been clearly established although we have been informed that one zoologist who was susceptible to the effects of the human *Ascaris*, experienced no discomfort after this susceptibility had been noticed in handling a large number of living specimens of the horse *Ascaris*. As noted by both Bastian and Goldschmidt the severity of the symptoms if originally mild may increase with repeated exposures, and in some cases the symptoms have become so severe as to force susceptible individuals to abandon further scientific work involving the use of the offending parasites.

The investigations that we have recently undertaken are the outcome of the discovery by one of us of his susceptibility to the toxic action of *Ascaris lumbricoides* on contact with the worm, (4) a discovery made about five years ago. At that time following the appearance of eye symptoms (irritation, lacrimation, and swelling of the eyelids) a cutaneous scratch test was made with some of the body fluid of the parasite, the results of which were positive. Later on one occasion the same individual experienced a severe general reaction as the result of a drop of *Ascaris* fluid falling unnoticed on a very small but fresh abrasion of the skin. This attack was characterized not only by a local wheal with surrounding erythema, but by extensive lymphatic involvement, which was evidenced by red streaks extending up the arm to the shoulder, swelling of the face, urticarial wheals over the entire body, very rapid heart, discomfort in the throat, and a sensation of warmth and breathlessness. The more alarming symptoms disappeared within a couple of hours, but the face and eyelids were still swollen the following day and the arm in the region of the local reaction was still swollen and reddened. More or less discomfort and various unpleasant symptoms have since been experienced under circumstances that involved direct or indirect exposure to *Ascaris*, although care has been taken to minimize the chances of close contact with the parasite so far as is possible in a parasitological laboratory.

In our recent investigations, which are still unfinished and of which a complete report can not now be given, certain observations have been made which appear to be of sufficient interest to warrant the presentation of a brief progress report.

About 20 persons, all white adults, have been tested for sensitiveness to *Ascaris lumbricoides* by means of a cutaneous scratch test. Among these there were 4 reactors, all of whom had been more or less exposed during periods of 5 to 25 years to contact with *Ascaris*. None of the reactors had a definite history of *Ascaris* infestation. Among the nonreactors were several who gave a history of having passed ascarids during childhood, and there were also several nonreactors who had at various times been considerably exposed to contact with *Ascaris* but had no recollection of unpleasant effects. Three of the reactors to the skin test had repeatedly experienced disagreeable symptoms on exposure to *Ascaris*; the fourth was previously unaware of his sensitiveness. In one of the cases the symptoms were asthmatic in type.

Different writers have reported that the characteristic odor of *Ascaris* may be detected on the breath of calves infested with *Ascaris vitulorum* and that the

meat of infested calves often smells so strongly of *Ascaris* as to render it unfit for food. In view of these reports the possibility naturally suggested itself that persons sensitive to *Ascaris* might also be similarly sensitive to pork from infested hogs if such meat contained toxic elements absorbed from the parasites infesting the animals. If it were found to be true that *Ascaris*-sensitive persons were sensitive to pork from infested hogs, the fact would be of great importance in its bearing on the question of pork sensitization, especially if at the same time such persons were insensitive to pork from noninfested hogs, inasmuch as a great deal of the pork that is eaten comes from *Ascaris*-infested hogs. Examination of about 2,500 hogs of various ages slaughtered in Chicago packing houses a few years ago showed an average of 41.1 per cent infested (3).

The following experiments were performed to determine whether pork from infested hogs contains a substance that will produce a skin reaction in persons sensitive to *Ascaris*. Aqueous, alcoholic, and ethereal extracts were made of pork from several infested hogs, and another similar series of extracts from a noninfested hog. Alcoholic and ethereal extracts were also made of fat from infested and noninfested hogs, respectively. None of these extracts gave a positive cutaneous scratch test in three *Ascaris*-sensitive individuals. Another experiment in which an aqueous extract of pork sausage was tested on six individuals, three of whom were sensitive to *Ascaris*, also gave negative results. The sausage used was a composite sample of sausage from several large meat-packing establishments. Any given lot of sausage manufactured in such establishments is likely to include trimmings from numerous hog carcasses, and in view of the frequent occurrence of *Ascaris* in swine the extract used in this experiment must have contained substances from the meat of a considerable number of infested hogs.

The results of these experiments, while not absolutely conclusive because of their negative character, indicate that the meat of *Ascaris*-infested hogs does not contain the substance that causes an urticarial skin reaction in *Ascaris*-sensitive individuals. In the light of this evidence there is no reason to suppose that *Ascaris* sensitization is involved in cases of pork sensitization.

Because of the writers' desire to learn something of the nature of the substance or substances in *Ascaris* that cause symptoms in susceptible persons, and, also, if possible, to isolate a substance that might appropriately be used to desensitize *Ascaris*-sensitive individuals, one of the writers undertook to separate various fractions of the constituent substances of the parasite. It was not considered advisable to attempt desensitization of human subjects with the crude substances of the worm inasmuch as certain pathological changes in the adrenals, thyroid, pituitary body, and pancreas of experimental animals have been recorded by other investigators following repeated injections of small quantities of the verminous material. Other investigators, also, notably Flury (1912), have reported the occurrence in *Ascaris* of a number of substances toxic to experimental animals. Flury (1) who has made the most extensive chemical and toxicological study of the parasite that has yet appeared has concluded that the toxic substances of *Ascaris* include aldehydes, free fatty acids, alcohols, esters, and two nitrogenous substances which are not protein in nature. He did not attempt to discover the substance which causes the urticarial skin reaction, and apparently was not familiar with this phenomenon. Shimamura and Fujii (1917) by fractionation of the watery extract from *Ascaris* separated a fraction which they termed "albumose-peptone" and to which they gave the name crude ascaron, applying the name ascaron to the inferred active principle, which they did not isolate (5). This ascaron they consider the active substance responsible for the toxicity of *Ascaris*, but their work throws little light upon the problem of the substance that causes the urticarial skin reaction.

Weinberg and Julien (6, 7, 8) have investigated the toxic action of the body-cavity fluid of the horse ascarid upon horses. The instillation of the fluid into the conjunctival sac was followed by a positive reaction in about two-thirds of 256 horses that were tested. This reaction which appears within a few minutes is characterized by edema of the eyelids, congestion of the conjunctiva, and lacrimation, occasionally accompanied by dyspnea, profuse sweating and diarrhea. Weinberg and Julien have concluded (8) that the toxicity of the fluid to sensitive horses is due to a number of active substances, inasmuch as they found the toxin to be thermostable, surviving exposure to a temperature of 120° C. for 20 minutes, filterable through a Chamberland filter, partially soluble in alcohol and in ether, and to contain volatile toxic constituents. The results that we have obtained in our investigations on human subjects with *Ascaris lumbricoides* are not entirely in accord with the conclusions expressed by Weinberg and Julien from the results of experiments with *Ascaris equorum* on horses. This discrepancy may be due not only to differences in the parasites and the experimental animals but also to the fact that Weinberg and Julien used body-cavity fluid of the worms in their tests while in our attempts to isolate an active substance we have been dealing with aqueous extracts of the worms and fractions of these extracts.

In our investigations swine ascarids (100 gm. in one lot and 1,774 gm. in another) after preliminary washing were ground up in a meat chopper, mixed with normal salt solution or 4 per cent ammonium sulphate solution and strained. The fluid portion was half saturated with ammonium sulphate and filtered. From the residue of this filtration a globulin fraction was obtained. The filtrate was precipitated by saturating with ammonium sulphate and after standing for 24 hours the albumens were collected by filtration. The filtrate was designated the protein-free filtrate for convenience although it still contained small amounts of proteins not precipitable by ammonium sulphate. It did not give the biuret reaction but gave a slight coagulum on heating. Three principal fractions were thus obtained from the original aqueous extract, a globulin fraction, an albumen fraction, and a so-called protein-free fraction. From the two latter various subsidiary fractions were prepared as shown in the list given below. Details of the chemical processes are reserved for a later report. The various fractions were tested on *Ascaris*-sensitive persons by means of the cutaneous reaction (scratch method). The results of these tests for each fraction are indicated by a plus or minus sign.

Fractions Nos. 1 to 4 were tested on three sensitive subjects, Nos. 5 to 9 on two, and Nos. 10 to 16 on one. In the test of fraction No. 8 the result was negative in the case of one subject and positive in the case of the other. The test was repeated in both cases with the same result, negative in one and positive in the other.

1. Globulin fraction.....	-
2. Albumen fraction.....	+
3. Protein-free filtrate (so-called).....	+
4. Protein-free filtrate heated just to boiling.....	+
5. Protein-free filtrate separated into approximately equal parts by distillation. Distillate.....	-
6. Same. Residue.....	-
7. Protein-free filtrate after air had been drawn through it for 55 hours.....	+
8. Protein-free filtrate treated with potassium permanganate.....	- (+)
9. Precipitate from acidified protein-free filtrate by Mayer's solution.....	+
10. Filtrate from acidified protein-free filtrate after treatment with Lloyd's reagent.....	-
11. Material recovered in weak alkali from Lloyd's reagent after action on acidified protein-free filtrate.....	+
12. Filtrate from acidified albumen fraction after treatment with Lloyd's reagent.....	-
13. Material recovered in weak alkali from Lloyd's reagent after action on acidified albumen fraction.....	-
14. Filtrate from albumen fraction after treatment with 50 per cent alcohol.....	+
15. Filtrate from albumen fraction heated 17 minutes in boiling water.....	+
16. Albumen fraction digested 36 hours with pepsin.....	-

From the results of these experiments, assuming for convenience of discussion that only one substance is involved, it is evident that the substance in aqueous extracts of *Ascaris lumbricoides* that causes the skin reaction in Ascaris-sensitive individuals is absent from the globulin fraction, present in the albumen fraction and present in the so-called protein-free filtrate, after the removal of the globulin and albumen fractions by precipitation with ammonium sulphate. It is weakened by oxidation with potassium permanganate so that it no longer produces a skin reaction in all Ascaris-sensitive persons. It is not volatile at temperatures between 20° and 100° C. It is thermolabile and is destroyed by exposure to a temperature of about 100° C. for less than an hour but may survive exposure to a temperature as high as 100° C. acting for a period of about 15 minutes. It is destroyed in the albumen fraction by digestion with pepsin. It is soluble in 50 per cent alcohol. From the acidified protein-free filtrate it is wholly adsorbed by Lloyd's reagent and wholly precipitated by Mayer's solution. It is also wholly adsorbed from the acidified albumen fraction by Lloyd's reagent but has not been recovered from the latter by subsequent treatment with weak alkali, a procedure which releases it from Lloyd's reagent after adsorption from the so-called protein-free filtrate. The question whether it is of protein nature has not yet been answered by our investigations.

In conclusion reference may be made to experiments which one of us has made with the body-cavity fluid and aqueous extracts of *Ascaris lumbricoides*, and extracts of the dried and powdered worms on various animals, by means of injections and local applications to the skin and eye. So far as concerns the local reactions produced in experimental animals which appear to be in a measure comparable to the urticarial reaction in human beings it is of interest to note that individuals sensitive to *Ascaris lumbricoides* may be found among both hogs (Pl. 1) and sheep. Local reactions have not been observed in guinea pigs or rabbits, which are relatively resistant to the toxic effects of Ascaris substance when injected subcutaneously, intraperitoneally, or intravenously, whereas sheep and hogs, particularly the former, are highly susceptible to the toxic action of Ascaris substance when injected parenterally. Some dogs will react to dog ascarids brought in contact with the conjunctiva, and Weinberg and Julien (6, 7, 8) as already noted have shown that horses commonly give an ophthalmic reaction to the body-cavity fluid of the horse ascarid.

Although the substances in *Ascaris lumbricoides* which cause local reactions in hogs and sheep may prove to be different from the substance that causes the local skin reaction in sensitive human subjects the fact that reactions very similar to that occurring in the human subject can be produced in lower animals as conveniently available as hogs and sheep promises to be helpful in the investigation of the question of Ascaris sensitization and utilization of these experimental animals for studies on Ascaris sensitization may lead to results having an important bearing on the general problem of sensitization of human beings to foreign substances.

SUMMARY

(1) Human beings are commonly sensitive to a substance contained in aqueous extracts of the nematode, *Ascaris lumbricoides*, an intestinal parasite of man and the pig.

(2) Sensitive individuals exhibit a positive skin reaction when this substance is applied to a scratch on the skin, similar to that exhibited in cutaneous tests for so-called foreign-protein sensitization.

(3) This substance is apparently absent from the meat of Ascaris-infested hogs and there is no reason to suppose that it is involved in cases of sensitization to pork.

(4) Cutaneous tests on sensitive human subjects made with various fractions of an aqueous extract of Ascaris material separated by chemical and physical

means have shown that the substance in this extract that causes the skin reaction is absent from the globulin fraction.

(5) It is present in the albumen fraction, and is also present in the filtrate after precipitation of the albumen and globulin fractions by ammonium sulphate.

(6) It is weakened by oxidation with potassium permanganate.

(7) It is not volatile at temperatures between 20° and 100°C.

(8) It is thermostable and is destroyed by exposure to a temperature of about 100°C. for less than an hour, but may survive exposure to a temperature as high as 100°C. acting for a period of about 15 minutes.

(9) It is destroyed in the albumen fraction by digestion with pepsin.

(10) It is soluble in 50 per cent alcohol.

(11) From the filtrate obtained after precipitation of the globulin and albumen fractions with ammonium sulphate it is wholly adsorbed in the presence of acid by Lloyd's reagent, and wholly precipitated by Mayer's solution.

(12) It is also wholly adsorbed from the acidified albumen fraction by Lloyd's reagent but has not been recovered from the latter by subsequent treatment with weak alkali, a procedure which releases it from Lloyd's reagent after adsorption from the aqueous extract from which the albumen and globulin fractions have been removed by precipitation with ammonium sulphate.

(13) The question whether the substance in *Ascaris lumbricoides* that causes the skin reaction in sensitive human subjects is of protein nature has not been answered by the investigations herein reported.

(14) A substance or substances that cause local reactions in some hogs and some sheep, comparable to the reactions observed in human subjects, are present in *Ascaris lumbricoides*. The ophthalmic reactions observed in these animals are altogether similar to those heretofore observed by Weinberg and Julien following the instillation of the body-cavity fluid of *Ascaris equorum* into the eyes of horses.

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PLATE 1

Ophthalmic reaction in a pig following the application of a few drops of *Ascaris* fluid to the conjunctiva. This animal also showed marked general symptoms in addition to the local eye reaction—vomiting, frothing at the mouth, frequent defecation, panting, and depression. The photograph was taken about 20 minutes after the application of the fluid.

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