A BLOOD-DESTROYING SUBSTANCE IN ASCARIS LUMBRICOIDES

[PRELIMINARY PAPER]

By Benjamin Schwartz

Zoological Division, Bureau of Animal Industry, United States Department of Agriculture

RELATION OF ASCARIS INFECTION TO ANEMIA

The view that parasitic worms secrete toxic substances which are absorbed by the host and which are responsible to a considerable extent for the symptoms which often accompany parasitic infections, has received strong support from experimental work with the body fluids and extracts of the various species of Ascaris that parasitize man and domesticated animals. Numerous experiments have shown that the body fluids and extracts of these worms produce decidedly harmful effects on susceptible animals into which they are injected. While it must be admitted that the effects of such injections are far more pronounced than the symptoms usually exhibited by animals that are known to harbor in their intestines numerous ascarids, experiments of this nature have thrown considerable light on the pathology of Ascaris infection, and have also added to our knowledge of the metabolic products of these parasitic worms.

That Ascaris may cause anemia is the opinion of various observers. According to Schimmelpfennig (9) and Weinberg and Julien (14), the post-mortem appearance of horses infected with ascaris suggests a condition of anemia. As far as man is concerned, a number of clinicians have emphasized the importance of Ascaris lumbricoides in relation to anemia. Thus, Demme (5) reports two cases of grave anemia in children, resembling pernicious anemia, which he attributed entirely to the presence of A. lumbricoides in the intestine. In one case death occurred, the cause of the disease not having been recognized, whereas in the second case complete recovery followed anthelmintic treatment. François (5) cites a number of cases of severe anemia resembling that of ancylostomiasis, in which A. lumbricoides was evidently the causal factor. Guiart (8) considers Ascaris to be a causal agent in anemia, ranking close to Dibothriocephalus and Ancylostoma in importance.

1 Reference is made by number (italic) to "Literature cited," p. 257-258

Journal of Agricultural Research, Washington, D.C. (253)
Vol. XVI, No. 9 Mar. 3, 1919
Key No. A-46
In view of the apparent importance of Ascaris as a cause of anemia, the possible relation between the secretions of the parasite and the anemia of the host has a high degree of practical interest. Certain investigators, notably Schimmelpfennig (9) and Flury (4), attribute hemolytic properties to the body fluids of Ascaris. Flury states, in fact, that the excretions of Ascaris when kept in vitro are hemolytic, and inclines to the view that anemia may be caused by the absorption of toxic substances produced by the worms. Weinberg (12, 13), Whipple (15), Alessandrini (7), and several other investigators, on the other hand, deny the presence of hemolytic substances in Ascaris and state quite emphatically that blood corpuscles of the host in contact with extracts of the worms remain intact. Recently Shimamura and Fujii (10), in a report of experiments with extracts of Ascaris on various animals, state that alcoholic and ethereal extracts of the parasites are hemolytic, but that watery extracts of the body substance of the worms previously freed from the ether and alcohol soluble portions produce no effect on red blood cells.

**SCOPE AND SUMMARY OF EXPERIMENTAL WORK**

For some time past the writer has been studying the problem of the possible absorption of toxic products by animals harboring ascarids. In this work A. lumbricoides of swine, of which an abundant supply is easily obtained, has been utilized. The experiments, the results of which are briefly summarized in this paper, were undertaken with a view of determining (1) whether the body fluids of the parasites are hemolytic, (2) whether the excretions of the worms kept in vitro contain blood-destroying substances, and (3) the relation which may exist between the anemia of ascariasis and the absorption by the host of toxic substances produced by the parasites. Sufficient data have already been accumulated to warrant certain conclusions, as follows:

(1) The body fluid of A. lumbricoides taken from worms shortly after their removal from the host is not hemolytic to the washed erythrocytes of swine, cattle, sheep, rabbits, guinea pigs, and rats.

(2) The fluid from worms which after removal from their host are kept alive in salt solution for a few days acquires hemolytic properties. Fluid from worms kept in vitro for 24 hours is only slightly hemolytic if at all, but fluid from worms kept under similar conditions from six to eight days is decidedly destructive to the red blood corpuscles of swine and sheep.

(3) The hemolytic property of the fluid is thermostable and is not destroyed by boiling.

---

1 Several investigators have shown that the fluid and extracts of human, horse, and swine ascaris have indistinguishable chemical and physiological properties.

2 Ascaris of swine is also referred to as Ascaris suum in order to distinguish it from the form which parasitizes man. The two forms are morphologically indistinguishable, however, so far as our present knowledge goes.
(4) There appears to be an inverse relation between the hemolytic property of the fluid and the presence of oxyhemoglobin in it. Fluid from fresh worms contains oxyhemoglobin and is nonhemolytic. When, however, the worms are kept alive in vitro, the oxyhemoglobin disappears from the fluid and can no longer be detected by spectroscopic examination one week after the worms have been removed from the host. Meanwhile the fluid becomes hemolytic. Whether oxyhemoglobin in itself is the sole factor in the inhibition of hemolysis or whether other substances are involved which are associated with the oxyhemoglobin and disappear simultaneously with it has not been determined.

(5) Salt-solution extracts of the worms made by grinding up 4 to 10 gms. of the fresh body substance of the parasites and suspending it in 100 cc. of an 0.85 per cent solution of sodium chlorid are hemolytic to the washed erythrocytes of swine and other mammals, the hemolytic potency of the extracts varying directly within certain limits with the duration of the extraction. The reaction is independent of the acidity of the solution, since it is not impaired by neutralization.

(6) Extracts of dried worms in an 0.85 per cent solution of sodium chlorid are decidedly hemolytic to the red corpuscles of various animals.

(7) Salt-solution extracts of the intestine of the worm are more destructive to blood corpuscles than extracts of the body wall, of the reproductive organs, or of the entire worm.

(8) The various salt-solution extracts also do not lose their hemolytic properties on boiling.

(9) The addition of blood serum to tubes containing a mixture of red blood corpuscles and body fluid or extract of the worms usually inhibits hemolysis.

(10) The hemolytic property of the fluid and of extracts of the worms can also be destroyed by the addition of a small quantity of laked blood.

(11) Excretions of the worms absorbed by the solution of sodium chlorid in which the parasites are kept in vitro are not hemolytic.

CONCLUSIONS

The failure to demonstrate hemolytic principles in the excretions of the worms when kept in vitro appears to favor the view that the hemotoxic substances of ascaris partake of the nature of endotoxins. There is also to be considered the possibility that the death of a worm in the intestine may be followed by a rapid disintegration of its tissues and the liberation of toxic substances before it passes out of the body of the host. Tallqvist (11), in fact, has shown in the case of another parasite (Dibothriocephalus latus) that the toxic substances are liberated only when the worm disintegrates, which affords a possible explanation why Dibothriocephalus sometimes produces no ill effects on its host, whereas in other instances a severe anemia is present. The fact that in some cases human beings and other animals infested with ascarids remain in apparent good health.
while in other cases they show evidences of suffering from such infestation may perhaps be explained in much the same way as the differences observed in cases of infestation with Dibothriocephalus.

The inhibitory effect of the serum on the hemolytic action of the body fluids and extracts of the worms appears to be a direct negation of the view that anemia of animals harboring ascarids is due to the toxic secretions of the worms. It is necessary to remember, however, that a reaction \textit{in vivo} may be very different from a reaction \textit{in vitro}.

Apart from the question of anemia as a result of the absorption of toxins produced by Ascaris, there is the question of anemia as a result of the direct abstraction of blood by the parasite. The opinion that Ascaris is a bloodsucker has been expressed by Schimmelpfennig (9), who based his view largely on the fact that the body fluid of ascaris contains oxyhemoglobin, the source of which presumably is the blood of its host. The view that Ascaris may suck blood is also supported by the structure of the mouth parts of the parasite and the lesions observed in the mucosa of intestines of animals harboring ascaris.

It should be remembered that ascaris is provided with strong chitinous lips, denticulated along their edges. That such buccal armature could succeed in lacerating the smaller blood vessels of the intestine is by no means improbable. Blanchard (2) states that there can be no doubt that \textit{A. lumbricoides} bites the intestinal mucosa. Guiart (7) has shown that \textit{A. conocephala} is often firmly attached to the mucosa of its host; he also states that Leroux found wounds in the intestinal mucosa of man resembling punctures which were apparently produced by \textit{A. lumbricoides}.

Friedberger and Fröhner (6) state that the intestinal mucosa of dogs harboring ascarids often shows evidence of punctures.

The above observations, coupled with the presence of oxyhemoglobin in the worms, a substance which apparently is constantly being excreted by the parasites (to judge from their behavior \textit{in vitro}) and which consequently must be as constantly renewed, appear to favor the view that Ascaris probably supplements its food intake by sucking blood from time to time. The hemolytic substance which is particularly abundant in the intestine of the worms apparently serves the purpose of liberating the oxyhemoglobin from the corpuscles some of which passes into the body fluid of the parasites. In this connection it should be recalled that Ascaris is rich in iron and that this substance enters in considerable quantity into the composition of the eggs (Schimmelpfennig, 9). The significance of the oxyhemoglobin in the body fluid of the worms is not well understood. Whether it merely represents a by-product in the metabolism of the worm and is always excreted as such, or whether it may also first be broken down into simpler compounds with the retention of some of the iron by the tissues of the parasite, still remains to be answered: Whether or not oxyhemoglobin fulfills an important function in the life processes of the worm—perhaps in oxidation—is another question to be solved. In this
connection it is interesting to observe that coincident with the disappearance of oxyhemoglobin from the worms \textit{in vitro} they become sluggish, and that their existence after the complete elimination of this substance is very brief.

\section*{LITERATURE CITED}

(1) \textsc{Alessandri}, Giulio.

(2) \textsc{Blanchard}, Raphaël.

(3) \textsc{Demme}, Rudolf.

(4) \textsc{Flury}, Ferdinand.

(5) \textsc{François}, E.

(6) \textsc{Friedberger}, Franz, and \textsc{Fröhner}, Eugen.
\textit{1895. Pathology and Therapeutics of the Domestic Animals. . . .} Translated from the most recent edition, with annotations by W. L. Zuill. Together with selections from the notes of the French translators, and also from those of Trasbot. 2 v. Philadelphia.

(7) \textsc{Guiart}, J.

(8) ———

(9) \textsc{Schimmelpffennig}, Gustav.

(10) \textsc{Shimamura}, Torii, and \textsc{Fujii}, Hajime.

(11) \textsc{Tallqvist}, T. W.

(12) \textsc{Weinberg}, M.
(13) Weinberg, M.

(14) ——— and Julien, A.
1913. RECHERCHES SUR LA TOXINE ASCARIDienne. In Hyg. Viande et Lait, ann. 7, no. 5, p. 225-244, 2 fig.

(15) Whipple, G. H.