THE PRESENCE OF A WEAK HEMOLYSIN IN THE HOOK WORM AND ITS RELATION TO THE ANEMIA OF UNCINARIASIS.[1]

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The anemia which is usually present in uncinariasis has been a favorite topic of discussion by all writers on the subject, and many explanations have been advanced. Perhaps the most popular theory depends on the assumption that the hook worm secretes some hemolytic principle which is absorbed by the host and destroys the red cells. The object of this paper is to show that the hook worm of man does contain a very weak hemolytic agent, active *in vitro*, but that this hemolysin is demonstrable only in concentrated extracts of the hook worm, and could have no relation to the secondary anemia of the host. The hemolysis takes place slowly, very little being recorded in six hours, and the readings were usually made after twenty hours. The hemolysin is present in all parts of the worm, and probably is related to its intestinal canal—a fact which excludes the possibility of any active hemolysin being secreted by the cephalic glands of the hook worm. The following fact goes to show that some hemolysin is present in the alimentary tract of the worm. Hook worms containing fresh blood removed from the mucosa, washed and placed in citrated saline, at once begin to pour out the intestinal contents from mouth and anus. This material is made up of cell detritus, intact red blood cells and *blood-stained* fluid. That we see many intact red blood corpuscles in the material passed out of the anus, speaks for a rapid ingestion and a weak hemolytic factor.

In a recent communication (8) on "Uncinariasis in Panama" I have reviewed the anatomical findings in a series of autopsies, recorded the frequency of hook worm infection, and noted some

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facts relating to the anemia of this disease. It has been shown that the hook worms ingest blood and epithelial cells from the mucosa and presumably digest both. Evidence has been brought forward to indicate that the anemia is due to two factors at least: (1) direct loss of blood through activity of the parasites; (2) a diffuse inflammation of the mucosa and submucosa of the jejunum caused by the bites of hook worms which damage the mucosa and give entrance to the intestinal bacteria. Attention has been called to the "*blood cysts*" which often contain hook worms. The lesion is due to erosion of a vessel by the hook worm in the submucosa giving rise to a definite hematoma. The hook worm may live in this blood clot for a few days as shown by the number and character of wandering cells in the margin of the clot. The worms when removed are always active, and show no signs of injury due to their stay in the blood of the host. Here we have an ideal experiment performed by the hook worm—the formation of a small sac of blood in the tissues of the host with an included parasite and all conditions favorable for the action of any hemolytic agent—yet we find very little solution of the red blood corpuscles. Study of such tissues shows many well-preserved red blood cells in all parts of the blood cyst, and very little evidence of hemolysis. It seems that this observation alone is sufficient to exclude the presence of any powerful hemolysin in the hook worms, and is a link in the chain of evidence that the hook worm does not secrete a hemolysin, which is absorbed by the host and gives rise to the secondary anemia.

It seems probable from this series of observations that the weak hemolysin found in the hook worm is present in the digestive tract, and possibly related to its digestion of the host's blood—a part of the parasite's food. The hook worms used in these experiments were taken from the intestinal mucosa at autopsy and used while actively motile: such material seems to be more valuable than that obtained from stools after the exhibition of thymol. If we compare the experiments in which human hook worms were used, with those in which dog hook worms were studied, we find that the results are similar—that both types of hook worm act on the blood of man and the dog. Parallel experiments with the whip worm and round worm of man act as excellent controls and show that

concentrated extracts of whip worms may be faintly hemolytic to some bloods and not to others. Emulsions of *Ascaris lumbricoides* are never hemolytic even when the worms are unwashed and carry numbers of intestinal bacteria into the mixtures.

Various experiments have been recorded to show the presence or absence of a hemolysin in various intestinal parasites. Loeb and Smith, working with *Ankylostomum caninum,* demonstrated the presence of a coagulation inhibiting substance in the cephalic glands and noted that in their mixtures there was no hemolysis in seventeen hours. Their method was very similar to that used in these experiments, but their emulsions were very much more dilute: about six or eight worms to one cubic centimeter of extract. In these experiments from twenty to fifty worms to one cubic centimeter of extract were used and the incubation was longer. Their experiments show how weak is this hemolytic factor.

Weinberg, working with *Sclerostomum equinum,* demonstrates a hemolysin which is active toward the blood of the horse and various other animals. It is more marked in the head of the worm, but is present in the digestive tract as well. It is quite resistant to heat and is easily soluble in saline. Other intestinal parasites of the horse show no such hemolysin (*Oxyuris equi, Ascaris megalocephala* and *Tenia plicata*).

Preti has studied the Old World hook worm (*Ankylostomum duodenale*) and states that he finds a hemolytic substance. This is not soluble in saline, but easily soluble in ether and alcohol. It is not destroyed by boiling for three hours. Trypsin frees the hemolysin and makes it soluble in water. In this preliminary communication he gives no information concerning his methods and controls, nor does he apply his methods to other intestinal worms. He makes no statement as to the rapidity of the hemolysis. He believes that the hemolytic substance is related to the group of lipoids and is very similar to the hemolysin isolated from *Bothriocephalus latus* by Tallqvist.

Many other writers (Ashford and King, Baker) cite the pigmentation of the liver and spleen, the hyperplasia of the marrow, and the advanced anemia as conclusive evidence of some hemolytic substance produced by the hook worms and absorbed by the host.

All these changes are found in secondary anemias caused, for example, by any long-continued bacterial infection of low grade, and evidence has been brought to show that there is in many cases of uncinariasis just such a low grade bacterial infection with subacute inflammation in the mucosa or submucosa. The increase of eosinophiles in the marrow and in the tissues close to the parasites is not peculiar to this disease, and is no argument in favor of a hemolysin.

HOOK WORMS OF MAN.

The first three experiments (Charts I, II, and III) can be compared as the same emulsions were used in each. Emulsion (1) of seventy New World female hook worms was made up in 3.5 cubic centimeters saline. Some of the worms were dead and the emulsion had a slight putrefactive odor. All emulsions were used after standing for thirty minutes, which gave opportunity for all particles to sediment out, leaving an opalescent supernatant fluid. Emulsion (2) of sixty Old World hook worms (male and female) was made up in 3.5 cubic centimeters saline; all worms were alive. These worms together are of greater bulk than those in Emulsion (1), and there is no odor. Emulsion (3) of thirty New World male hook worms was made up in 3.5 cubic centimeters saline.

CHART I.

Citrated Blood in Uniform Dilution (Human).

Hook worm emulsions.	Black male, 20 years.							White male, 25 years.							
(1) In 3.5 c.c. saline. 70 new world females.	4				4			4					4		
(2) In 3.5 c.c. saline. 60 old world worms.		4	10			4			1	4	15			4	
(3) In 3.5 c.c. saline. 30 new world males.				4			4					4			4
Hemolysis.	1	2.5	3.5	1	1	1.5	1	2	0.5	2	3	2	2	2.5	2
Readings after 22 hours.					Incubated 6 hours at 38° C.								Incubated 6 hours at 38° C.		

Figures indicate number of drops of emulsion added to citrated blood.

The above charts (I, II and III) show that there is a weak hemolytic agent present in both Old and New World varieties of hook worms—in both males and females. It acts slowly and there is scarcely any hemolysis noted in six hours. Incubation at 38° C. has the slightest or no effect. The hemolytic agent acts on the blood of man and of the dog and rat.

CHART II.

Human Citrated Blood.

Hook worm emulsions (as in Chart I).	White male, 45 years.											
(1) 70 new world female worms.	4	15	15 sed.	23								
(2) 60 old world hook worms.					4	15	15 sed.	24				Controls.
(3) 30 new world male worms.									5	15	25	
Hemolysis.	1	1	1	2	0.5	1	3	2	0.5	0.5	1	0

Readings after 22 hrs.; (sed.) indicates the fine sediment which settles out from any emulsion after standing.

CHART III.

Animal Citrated Blood.

Hook worm emulsions (as in Chart I.).	Mongrel active pup.								Adult white rat.							
(1) 70 new world females in 3.5 c.c. saline.	4				4			Controls.	1	4	15			4		
(2) 60 old world worms in 3.5 c.c. saline.		4				4						4			4	
(3) 30 new world males in 3.5 c.c. saline.			4	15			4						4			4
Hemolysis.	1	1.5	1	1.5	1	1	1	0	0.5	1	2	1	0.5	1	2	0.5
Readings after 22 hours.				Incubated 6 hrs. at 38° C.												

The emulsions used in Experiment 4 (Chart IV) were made as usual. The active hook worms were cut in two, leaving about one-fifth of the worm in the head end. The œsophagus of the worm is easily visible, and the cut was made about 0.5–1 mm. posterior to the œsophagus. Emulsions were placed in ice box over night and the clear supernatant fluid used, except where marked (sed.), indicating the use of the finely granular sediment.

Chart IV shows that the hemolytic factor is present in all parts of the hook worm, in the head end as well as in the body, but more marked in the larger body portion. The clear supernatant fluid is hemolytic as well as the granular sediment of the emulsions. The whip worm emulsion is not hemolytic to the blood of these two persons.

Experiment 5.—The worms were cut in two exactly as in Experiment 4. The worms were all alive and active when used except in (5) where they had been dead for twenty-four hours. Emulsion (5) of forty dead hook worms contained both Old and New World types.

CHART IV.

Citrated Blood in Uniform Dilution (Human).

Hook worm emulsions.	White male, 45 years.											Black male, 20 years.			
(1) Bodies of 25 large old world hook worms in 1.5 c.c. saline.	10			Control.	10			Control.	10 sed.			10			Control.
(2) Heads of 25 large old world hook worms in 1 c.c. saline.		10				10				10 sed.			10		
(3) Emulsion of 3 large whip worms in 1.5 c.c. saline.			10				10				10 sed.			10	
Hemolysis.	3.5	1.5	1	1	2	trace	0	0	2	0.5	0	1	1	0	0
Readings after 22 hrs.	Incubated at 38° C. for 22 hrs.								(Sed.) — Indicates the use of finely granular sediment in the emulsions.						

Figures indicate number of drops of emulsion added to citrated blood.

The emulsions were all placed on ice over night and when used all were odorless except (5) which showed a strong putrefactive odor. The supernatant fluid was slightly opalescent. The sediment was abundant and rich in sand which had been added in greater amounts than usual.

CHART V.

Citrated Blood in Uniform Dilution.

Hook worm emulsions.	White male, 25 years.												Small anemic pup.					
(1) 82 new world female (bodies) in 2.5 c.c. saline.	10					Control.	10					Control.	10					Control.
(2) 82 new world females (heads) in 2 c.c. saline.		10						10						10				
(3) 77 new world males in 2.3 c.c. saline.			10						10						10			
(4) 17 old world worms in 2 c.c. saline.				10						10						10		
(5) 40 dead hook worms in 2 c.c. saline.					10						10						10	
Hemolysis.	0.5	3	0.5	1	3	trace	0.5	2.5	0.5	3	3	0.5	3	3	3	4	5	1
Readings after 24 hrs.	Supernatant fluid.						Sediment used was very rich in sand.											

Figures indicate number of drops of emulsion added to citrated blood.

Chart V shows that the hemolytic agent is present in both body and head parts of the hook worms (see Chart IV) in both males and females. The

supernatant fluid and sediment are both hemolytic. The dead worms are slightly more hemolytic than the live ones, and this is possibly due to the putrefactive products. Emulsions of human hook worms will hemolyse human and dog blood. The pup's blood used in this experiment was always very sensitive to any hemolytic agent.

Experiments 6 and 7 are very similar. Emulsion (1) was made as usual from Old World hook worms (males and females); there was greater concentration in Experiment 6. Pancreatic extract (2) was made as follows: a dog's pancreas was freed from fat, finely divided and ground up with sand to a thick paste; 12 grms. of this material was placed in a flask and 70 c.c. of distilled water and 3 c.c. of chloroform were added. The mixture was incubated for twenty-four hours at 38° C. This fluid digested coagulated egg albumen, but was not very active at room temperature. Solution of sodium carbonate (3) was quite dilute, but its strength was not accurately determined; it gave a definite blue reaction on litmus.

CHART VI.

Human Citrated Blood.

	White male, 18 years.								
(1) 30 old world hook worms in 1.4 c.c. saline.	5	10	15 sed.	5	5	5			Control.
(2) Pancreatic extract (dog).				1	5			5	
(3) Sodium carbonate in dilute solution.						5	5		
Hemolysis.	1	1.5	3	1.5	1.5	1	0	2	faint trace.
Readings after 22 hours.									

Figures indicate number of drops of emulsion added to citrated blood.

CHART VII.

Human Citrated Blood.

	White male, 22 years.								
(1) 44 old world hook worms in 2.5 c.c. saline.	5	10	15	Control.	10 sed.	15	15	15	Control.
(2) Pancreatic extract (dog).							10		
(3) Sodium carbonate in dilute solution.								10	
Hemolysis.	0.5	1	1	0	3	3	2	2	faint trace.
Readings after 18 hours.					Incubated at 36° C. for 18 hrs.				

The Charts VI and VII show that the hemolytic factor present in the Old World hook worm is uninfluenced by pancreatic extract or a weak solution of sodium carbonate. Incubation increases the hemolysis in small degree.

Experiments 8 and 9 must be compared as they both show the results of boiling, which destroys the hemolytic factor both in the human and the dog's hook worm.

Experiment 8.—Emulsion of 90 hook worms (Old and New World varieties) was made up in 3 c.c. of saline and divided into the three equal parts indicated in Chart VIII.

CHART VIII.

Citrated Blood in Uniform Dilution.

Emulsion of 90 hook worms in 3 c c. saline.	White male, 18 years.														Strong active pup.				
(1) 1 c.c. used fresh.	10			15	5 sed.				5						10			10 sed.	
(2) 1 c.c. in boiling water bath for 15 minutes.		10				10 sed.		Controls.		5		10 sed.		Controls.		10			Controls.
(3) 1 c.c. in boiling water bath for 60 minutes.			10				10 sed.				5		10 sed.				8		
Hemolysis.	1	0	0	2	2	0	0	0	1	0	0	0	0	0	1	0	0	1	0
Reading in 30 hrs.									Incubated 20 hrs. at 38° C.										

Figures indicate number of drops of emulsion added to citrated blood.

Chart VIII shows that boiling for fifteen minutes or over completely destroys the hemolytic agent. The hemolytic factor present in the human hook worm acts on dog's as well as on human blood (see Chart IX).

CHART IX.

Citrated Blood in Uniform Dilution.

Emulsion of 60 dog hook worms in 2 c.c. saline.	White male, 21 years.									Small anemic pup.				
(1) 1 c.c. used fresh.	10	Control.		10	10*	15*	Control.	10		Control.	10		10[1]	15[1]	Control.
(2) 1 c.c. placed in boiling water bath for 3 minutes.			10						10			10			
Hemolysis.	0.5	0	0	2	0.5	1.5	0	2	0	0	2	0.5	2	2	0
Reading after 20 hrs.			Incubated at 36° for 16 hrs.								Incubated at 36° C. for 16 hrs.				

Figures indicate number of drops of emulsion added to citrated blood.

* Sediment taken up in 1 c.c. saline, well mixed and used at once.

Hook Worm of Dog.—The experiments with *Ankylostomum caninum* were performed in precisely the same manner as were those with the hook worm of man, and usually at the same time. All conditions were equal and we may compare the results which are very similar.

Experiment 9 (compare Chart VIII).—Emulsion of 60 dog hook worms (males and females in about equal numbers) made up in 2 c.c. saline and divided into equal parts: (1) used fresh. (2) 1 c.c. of the turbid fluid placed in a boiling water bath for three minutes. Solution was placed on ice for one hour and then used.

Chart IX shows that the dog hook worm contains a hemolytic factor which is active towards human as well as dog blood. Boiling for 3 minutes destroys practically all of the hemolysin. The sediment taken up in saline and used at once shows that not all the hemolytic agent is extracted in the first emulsion.

Experiment 10 (compare Experiments 4 and 5).—One hundred hook worms (*Ankylostomum caninum*) were cut in two as in Experiment 4. The "head end" contains about one-fifth of the worm, including the cephalic glands, and the "body" contains the remaining four-fifths of the worm. Sodium carbonate solution (3) is that used in Experiments 6 and 7.

CHART X.

Citrated Blood in Uniform Dilution.

	Male adult dog.					Negro male, 25 years.								White male, 45 years.							
(1) 100 *heads* in 2 c.c. saline. Dog hook worms.	3		10		Controls.	3		10		10			Controls.	3		10		10			Controls.
(2) 100 *bodies* in 2.5 c.c. saline. Dog hook worms.		3		10			3		10		10				3		10		10		
(3) Sodium carbonate weak solution.										5	5	5					•	5	5	5	
Hemolysis.	trace	0.5	0.5	1	0	0	0.5	0.5	0.5	0.5	0.5	0	0	0	0	0.5	0.5	trace	0.5	0	0
Reading after 16 hrs.	No incubation.																				

CHART XI.

Human Citrated Blood.

	White male, 21 years.						
(1) Emulsion of 5 whip worms in 1.2 c.c. saline.	5	5	10	5 sed.	3 sed.		Control.
(2) Weak solution of sodium carbonate.				5	6	6	
Hemolysis.	0	1	1	0.5	0	0	trace
Readings in 18 hours.	All mixtures faintly alkaline to litmus.						

Figures indicate number of drops of emulsion added to citrated blood.

Chart X shows that Ancylostomum caninum, like the human hook worm, contains a hemolytic agent in all parts of its body, which is active for the blood of man and of the dog. Sodium carbonate in weak solution has no effect on the hemolysis. The larger body fraction seems to have a little more activity than the head fraction.

Whip Worms of Man.—Experiments 11 and 12 are very similar. Emulsion (1) was made with large active whip worms.

CHART XII.

Human Citrated Blood.

	White male, 22 years.					
(1) Emulsion of 5 large whip worms in 1.5 c.c. saline.	10	15	15			Control.
(2) Sodium carbonate dilute solution.			10	20		
Hemolysis.	0.5	2	1.5	0		0
Reading after 22 hours.		Incubated at 36° C.				

Charts XI and XII show that concentrated saline extracts of the human whip worm may contain a very weak hemolytic agent active toward the blood of some persons. It is uninfluenced by a weak solution of sodium carbonate.

CHART XIII.

Human Citrated Blood.

	White male, 18 years.						
Emulsion of 8 whip worms in 2 c.c. saline.	10	10	Control.	10	10	10	Control.
Weak solution of sodium carbonate.					5	5	
Hemolysis.	0.5	1	0	3	3	3	0
Readings in 18 hours.			Incubated at 37° C. for 18 hours.				

CHART XIV.

Human Citrated Blood.

	White human, 25 years.											
Emulsion of 10 whip worms in 2 c.c. saline.	5	10	15	10 sed.	15 sed.	Control.	5	10	15	10 sed.	15 sed.	Control.
Hemolysis.	0	0	0	0	0	0	0	0	0	0	0	0
Readings after 24 hours.							Incubated at 38° C. for 7 hours.					

Experiments 13 and 14 may be contrasted, as the emulsions are very similar, yet the blood of one case is hemolyzed and the blood of the other resists hemolysis. The blood used in Experiment 14 was always resistant to hemolytic agents.

Experiments 11–14 (see also Experiment 4) indicate that the human whip worm may contain a weak hemolytic agent, active toward some solutions of blood and not towards others. This action may be increased by incubation, but is unaffected by sodium carbonate in weak solution.

CHART XV.

Human Citrated Blood.

Emulsions of female *Ascaris lumbricoides.*	White male, 45 years.													
(1) Head — 1 cm. in 2 c.c. saline.	5	10					Control.	15 sed.			15			
(2) Body — 1 cm. in 2.5 c.c. saline.			5	10					15 sed.			15		Control.
(3) Tail — 1 cm. in 2.5 saline.					5	10				15 sed.			15	
Hemolysis.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Readings after 24 hours.											Incubated at 36° C. for 24 hours.			

Figures indicate number of drops of emulsion added to citrated blood.

CHART XVI.

Human Citrated Blood.

Emulsions of female *Ascaris lumbricoides.*	Black male, 25 years					
(1) Head end (1 cm.) *not* washed in 2.5 c.c. saline.	51	15 sed.				
(2) Tail end (1 cm.) washed in 2.5 c.c. saline.			10	15		Control.
(3) Body (1 cm.) washed, incubated — 45 mm.					15	
Hemolysis.	0	0	0	0	0	0
Readings in 27 hours.						

Round Worm of Man.—The last two experiments (15 and 16) are very much alike in all respects. In Experiment 15 a medium-sized female, *Ascaris lumbricoides,* was thoroughly washed in water and saline. For Emulsion (1) the head end was cut off (1 cm. in length) and ground up with sand and saline added

to 2 c.c. For Emulsion (2) a piece was cut out of the middle of the worm, a little less than 1 cm. in length, and made up in 2.5 c.c. saline. For Emulsion (3) about 1 cm. of the tail end was made up in 2.5 c.c. saline.

Experiment 16 was identical with Experiment 15 except that the head end (1) was cut off before the worm was washed and this emulsion was rich in intestinal bacteria. Emulsion (3) of body was incubated at 38° C. for forty-five minutes before use.

Charts XV and XVI show that the round worm of man (Ascaris lumbricoides) contains no hemolytic agent in any portion of its body.

Method.

Emulsions in all cases were made as follows: the hook worms were washed thoroughly in running water; then in normal saline solution. The living worms alone were used unless otherwise stated. In almost all cases the worms were studied and identified, as indicated in each experiment. The worms were ground up in a small mortar with clean, white sand and a few drops of saline added; then the grinding was continued until a thin, uniform, gray paste was formed. To this was added a measured quantity of 0.7 per cent. saline solution (1–5 c.c.) as is indicated in the charts. These turbid mixtures were placed on ice for a varying length of time. Unless otherwise stated the supernatant fluid which was usually opalescent was added to the blood.

The citrated blood was prepared in the same manner in all cases. Small test tubes usually used in hemolytic work, were half filled with citrated saline solution (7 grms. sodium chloride and 15 grms. sodium citrate dissolved in 1 liter of distilled water). To this was added three large drops of blood usually obtained from the ear thoroughly cleansed with alcohol. This gave a dilution of blood best suited for the work—about one part blood to eight or ten parts citrated saline. After addition of the emulsion by a long dropper, the tubes were inverted several times to get a uniform mixture. The majority of the tubes were left at room temperature which was quite constant at Ancon (75°–85° F.). Other tubes were incubated in the thermostat. Hemolysis was estimated by the writer in all cases and is expressed arbitrarily by numbers, considering complete hemolysis as 10. The estimation was done carefully, and we feel that the relative values or amounts of hemolysis are fairly accurate. The charts are all made on the same plan as Chart I. The citrated blood is of constant dilution—human or animal as indicated at the top of the chart. The figures in the upper columns indicate the number of drops of the emulsion added to the citrated blood, and the resulting hemolysis is expressed by figures in the bottom column.

SUMMARY.

1. The hook worm of man—both the Old and New World types—contains a weak hemolytic agent active *in vitro*. It is soluble in salt solution, is easily destroyed by heat and acts slowly.

2. The hemolysin is present in all parts of the worm and probably is associated with the intestinal tract.

3. The hook worm of the dog contains a similar hemolysin.

4. These hemolysins are not specific, but will act on human blood as well as on that of the dog and rat. They are only demonstrable in concentrated extracts.

5. Concentrated extracts of the human whip worm may be hemolytic to some bloods.

6. The round worm of man contains no hemolytic principle in any part of its body.

7. It seems very unlikely that this weak hemolysin found in the hook worm can have any relation to the anemia of uncinariasis.

8. Study of the "blood cysts" in the human intestine shows that the hook worm may live in a small amount of the host's blood for days without causing any marked hemolysis.

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