HEMOTOXINS FROM PARASITIC WORMS

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I. INTRODUCTION

Aside from the purely mechanical injuries which parasitic worms may inflict upon their host as a consequence of their migrations, displacement of a certain amount of the host's tissue, bites and laceration of the mucosa, obstruction of ducts, and various other mechanical disturbances, it has been generally assumed that they may also produce harmful effects as a result of their toxic secretions. Despite the fact that the data on which the view that parasites secrete toxic substances is based, so far as they have been recorded heretofore in the literature, are somewhat contradictory, they have been accepted by a large number of investigators as affording a more plausible explanation of the chemical pathology of helminthiasis than the data with reference to any other theory that has thus far been advanced. With reference to the subject of toxic products of parasitic worms, Wells (1918) states:

The subject has received much less consideration than its importance deserves, as we are quite in the dark as to how much of the effects produced by animal parasites are not merely mechanical, but are due to soluble poisons that they secrete or excrete. Some of the parasites probably cause harm mechanically and in no other way, but with most of them there is more or less evidence of the formation of poisonous substances.

While it must be admitted that the evidence in favor of the view that parasites secrete products toxic to the host is as yet rather incomplete, the fact of the existence of such toxic products can not be denied. So far as they have been investigated, the serological reactions of hosts harboring parasites afford proof that parasitic worms liberate products against which the host develops defense or “immunity” reactions. It has been known for a relatively long time that in cases of infestation with species of Trichinella, Schistosoma, Necator, and Anyclostoma a

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1 Resigned December 15, 1920.
2 Dates in parenthesis refer to “Literature cited,” p. 428-432.
high eosinophilia is commonly present. An increase in the number of eosinophile leucocytes has also been observed, although not as regularly, in cases of infestation with species of Ascaris, Oxyuris, Strongyloides, and other nematodes. Similar conditions have also been encountered in cases of infestation with *Taenia solium*, *T. saginata*, *Fasciola hepatica*, *Clonorchis sinensis*, and other cestodes and trematodes. As a matter of fact, eosinophilia is so commonly associated with parasitic infestation that the finding of a high eosinophile content in the peripheral blood is generally considered as presumptive evidence of parasitic infection. In a recent extensive review of the literature on the subject of eosinophilia, Schwarz (1914) states that an increase in the number of eosinophile leucocytes in the peripheral circulation in cases of parasitic infestation is, from an etiological viewpoint, the most clear-cut illustration of general eosinophilia.¹

Aside from the cellular immunity reactions, as shown by the increase in the number of eosinophile leucocytes in the blood in cases of infestations with parasitic worms, there appears to be evidence of a humoral immunity as well. In the case of hydatid (*Echinococcus*) disease of man and animals, it has been shown by a number of investigators that specific antibodies are present in the blood of the host, demonstrable by the technic of complement fixation and precipitate formation. That such immunity reactions are not limited to hydatid disease is the opinion of certain investigators, who support their views by experimental evidence which shows that specific antibodies are also present in cases of infestations with species of Ascaris, Fasciola, Schistosoma, and other parasitic worms.²

The facts cited in the preceding paragraphs appear to indicate that hosts harboring parasitic worms develop typical defense or "immunity" reactions to the absorption of foreign and presumably toxic substances of parasitic origin. A logical corollary to the study of the serological reaction of animals to secretions of parasitic worms is the study of the secretions themselves with reference to their chemical and physiological properties. This subject has recently received considerable attention in studies on the causes of pernicious or infectious anemia of horses, a disease of unknown etiology, which Seyderhelm and Seyderhelm (1914) attribute to a secretory product of an internal parasite (the larvae of *Gastrophilus*). Although the assumption of the Seyderhelms has not been confirmed, numerous experiments by different investigators have shown that injection into animals of extracts of various parasitic worms may lead to serious consequences, frequently terminating in death. Despite the fact that these experiments are in a general way confirmatory of the work of earlier investigators on the physiological effects of extracts of

¹"Die Vermehrung der α-Zellen in peripheren Blut bei Anwesenheit von Parasiten aus dem Stamme der Würmer ist vielleicht die ätiologisch am meisten klarzustellte Form der allgemeinen Eosinophilie" cited in G. Ghedini's article.

²References to and a summary of this phase of the subject may be found in an article by G. Ghedini.
parasitic worms, the experimental evidence on the subject is somewhat contradictory, due no doubt to the fact that different investigators experimented under different conditions. The study of the effects of extracts of parasites on living animals presents numerous difficulties and complications and may lead to contradictory results unless suitable precautions are taken to control various extraneous factors. More accurate studies on the effects of toxic products may be carried out in vitro, provided the toxic substance in question has affinity for tissues and cells available for such experiments. As is well known, red blood cells serve as excellent indicators of test-tube reactions in which hemotoxic substances are involved, and in the case of toxic products of parasitic origin, experiments with red blood cells are of great importance in view of the fact that in many parasitic infestations anemia is a characteristic symptom. The effects of extracts of parasitic worms on red blood cells, especially of extracts of those parasites that are known to cause anemia, are thus of interest with reference to the possibility that the parasites in question secrete specific toxins for the blood (hemotoxins).

II. REVIEW OF LITERATURE ON HEMOTOXINS IN PARASITIC WORMS

The same year in which Ehrlich (1898) announced the discovery of a blood toxin produced by Bacillus tetanus, Schaumann and Tallqvist (1898) reported the discovery of a blood toxin in the broad tapeworm of man (Diphyllobothrium latum). Ehrlich's discovery in the field of bacteriology served as a stimulus to the study of bacterial hemolysins by various investigators and was followed by a series of investigations into the nature and action of these hitherto unknown products of bacterial growth. Although the discovery of Schaumann and Tallqvist did not arouse the same degree of activity in parasitology as Ehrlich's discovery aroused in bacteriology, the results of their investigations were not without influence on subsequent researches in parasitology, the influence being especially marked in connection with studies on the causes of the anemia that occurs in cases of infestation with hookworms.

The facts published by Schaumann and Tallqvist (1898) may be briefly summarized as follows:

Macerated material of Diphyllobothrium latum contains a hemolytic substance active in vitro as well as in vivo. Peptic digestion liberates the hemolysin from the tissues of the parasites. The introduction of D. latum material into dogs parenterally or per os leads to a marked reduction in the number of erythrocytes.

In a later paper Tallqvist (1907) gives a more detailed account of the nature of the hemotoxic secretions of Diphyllobothrium latum. The hemolytic principle is closely bound to the cells of the parasite and is but slightly soluble in water and physiological salt solution. By means of peptic digestion and alcohol or ether extraction it becomes dissociated from the tissues and goes into solution. The hemolysin is thermostable and does not cause the development of antibodies when injected into animals. In these respects it resembles normal tissue hemolysins. Tallqvist found, moreover, that D. latum contains not only a hemolysin but also a hemagglutinin. The latter is soluble in water in contrast to the water-insoluble lipoidal hemolysin. The hemagglutinin as well as the hemolysin is nonspecific. The potency of these agents varies, however, for different species of red blood corpuscles.

Faust and Tallqvist (1907) studied the Diphyllobothrium hemolysin as to its chemical nature. These investigators found that extraction of the entire worm material in ether removed all the hemolysin from the tissues of the parasite, since the removal of
the ether-soluble fraction left a fraction entirely devoid of hemolytic activity. The ether-soluble fraction was then freed from its lecithin and cholesterol content without injuring its hemolytic activity. In the remaining ether fraction (freed from lecithin and cholesterol) Faust and Tallqvist identified three fatty acids, namely, palmitic, stearic, and oleic acids. The first two substances did not exhibit any hemolytic properties, whereas oleic acid was found to be markedly hemolytic. These investigators therefore concluded that oleic acid is the active principle of Diphyllobothrium hemolysin.

In a later paper Faust (1908) records the results of experiments on the effects of oleic acid on dogs when introduced per os. In brief, this investigator concluded that prolonged feeding of oleic acid to dogs produced anemia in the latter, as evidenced by a reduction in the number of red blood corpuscles. Beumer (1919), however, has found, on the contrary, that animals may be fed daily with considerable quantities of oleic acid for long periods without permanent ill effects, and has failed to substantiate the harmfulness of oleic acid.

In this connection it is of interest to recall the experiments of Dascotte (cited by Weinberg, 1912), who states that extracts of Taenia solium and T. saginata, cestodes parasitic in man, dissolve human red blood corpuscles. Dascotte found, moreover, that the hemolysin from these parasites is soluble in alcohol and resistant to heat at temperatures of 100° to 120° C. Calamida (1907) found that extracts of two species of cestodes from carnivores (Dipylidium caninum and Multiceps multiceps) are hemolytic to the red blood corpuscles of rabbits and guinea pigs and that the hemolysin goes through the pores of a Berkefeld filter. According to Weinberg (1907), physiological salt-solution extracts of two species of tapeworms parasitic in horses (Anoplocephala picata and A. perfoliata) have no deleterious effects on the blood corpuscles of the horse. Tallqvist (1907), on the other hand, denies the presence of hemolysins in cestodes other than Diphyllobothrium latum. He states that he worked with a number of species, including T. saginata. He admits that he sometimes observed slight hemolytic effects of extracts of these parasites but expresses the opinion that they are to be ascribed to secondary degeneration products associated with acid formation.

While Diphyllobothrium latum is capable of causing severe anemia in man, clinically indistinguishable from pernicious anemia and, according to many investigators, differing from the former in one respect only, namely, by the disappearance of the symptoms and recovery of the patient after expelling the parasite, there are numerous cases on record in which the presence of D. latum in man was not accompanied by anemia. In fact, grave blood disturbances in cases of D. latum infection are, according to the observations on record, not as common as the incidence of infection with this tapeworm, a fact which has given rise to considerable speculation as to the causes of the occasional appearance of anemia in the course of infection with this parasite. These speculations will be referred to elsewhere in this paper.

In contrast to the occasional appearance of anemia in cases of infection with Diphyllobothrium latum infections with hookworms (Necator and Ancylostoma) are generally accompanied by severe anemia. That the causes of anemia in hookworm disease are due to a toxin is a view which was adopted by a number of investigators on a purely a priori basis, because the direct abstraction of blood by these parasites, even when present in large numbers, fails to account for the severity of the clinical picture usually present in such cases. This fact was recognized early in the study of the disease, and led to the postulation of the "toxin theory."

Luscana (1890) found that as a result of injecting rabbits with urine taken from patients suffering from hookworm disease, the animals developed symptoms of anemia. It was not until 1905, however, that the toxin theory received more direct experimental support from Calmette and Breton. These investigators found that salt-solution extracts of the Old World hookworm of man (Ancylostoma duodenale) are hemolytic to the red blood cells of man. Alessandrini (1904) had already found by direct microscopic
observation that human red blood corpuscles are destroyed when placed in contact with the cervical glands isolated from hookworms (species not given but presumably *A. duodenale*), but subsequent investigation showed that the hemolysin is not limited to the cervical glands.

Loeb and Smith (1904) in the course of experiments with salt-solution extracts of the dog hookworm (*Ancylostoma caninum*), found that these extracts showed no hemolytic properties and left the blood still intact and uncoagulated after being in contact with it for 17 hours. Reference to the work of these investigators on the anticoagulating property of hookworm extract (*A. caninum*) will be made elsewhere in this paper.

Liefmann (1905) found that in two out of three experiments salt solution extracts of *Ancylostoma caninum* produced slight hemolysis of dog blood. This writer observed intact erythrocytes in the intestines of the worms and therefore came to the conclusion that the parasites do not secrete a hemolysin. Liefmann fails to state whether or not he washed the blood corpuscles before testing them against hookworm extract.

Preti (1908) found that the Old World hookworm of man (*Ancylostoma duodenale*) contains a hemolysin insoluble in salt solution but soluble in ether and alcohol. He states that tryptic digestion liberates the hemolysin and renders it soluble in water. He found the hemolysin to be resistant to boiling for three hours and nonspecific, since it was equally potent against the blood corpuscles of several other species of animals as well as man.

In the course of his investigations concerning ancylostomiasis and beriberi, Noc (1908) found that physiological salt-solution extracts of the hookworm of man (*Necator americanus*) are hemolytic to the washed red blood corpuscles of man. He states that the hemolysin withstands a temperature of 80° C. for one hour without injury to its potency. Noc found that whereas the blood serum of patents suffering from severe ancylostomiasis and beriberi contained no antihemolysins, that of normal persons and of those recovering from these diseases was antihemolytic.

De Blasi (1908) examined the blood serum of 12 human subjects infested with hookworms (*Ancylostoma duodenale*) and found that after the serums were heated for 30 minutes at 56° to 62° C. they acquired hemolytic properties. Before heating, the serums in question were not hemolytic. Heating the serum evidently destroyed some antibodies which neutralized the potency of the hemolysin. The heated serum of normal persons, according to this writer, did not contain any hemolysins.

Whipple (1909) records tests of salt-solution extracts of *Ancylostoma caninum*, *A. duodenale*, and *Necator americanus* on unwashed citrated blood of man, dog, and rat. He states that he found a weak hemolysin in the three species of hookworms exhibiting similar properties, namely, nonspecificity, susceptibility to boiling which destroys it, and distribution in all parts of the body of the worms. According to Whipple, the hemolysin is only demonstrable in concentrated extracts, and probably bears no relation to the secondary anemia of ancylostomiasis.

Loeb and Fleisher (1910) state that a salt-solution extract of *Ancylostoma caninum* containing as much as 5 mgm. of the powdered worm material in 1 cc. of salt solution did not produce any hemolytic effect on the washed erythrocytes of the dog. These writers also state that lecithin used in doses in which it alone produced no hemolytic effect failed to activate *A. caninum* extract. Loeb and Fleisher admit the possibility that the temperature at which the specimens were dried (42° to 50° C.) may have had an injurious effect on the hemolysin, but they do not consider this very probable.

Recently Usami and Mano (1919) have studied the effects of hookworm extracts on red blood cells. These writers state that hookworm hemolysin is thermostable, insoluble in water, and soluble in alcohol, ether, and acetone.

It will be seen from the foregoing summary with reference to hookworm extracts that Loeb and Smith (1904) and Loeb and Fleisher (1910) are the only investigators who failed to observe hemolysis in the presence of these extracts. As will be shown elsewhere in this paper, the negative results of Loeb and Smith may have been due
to the antilytic action of normal blood serum. The negative results recorded by Loeb and Fleisher (1910) may have been due to insufficient or faulty extraction of the worm material, insufficient quantity of powder used in the experiments, or possibly to the destruction of the hemolysin by drying at temperatures between 42° and 50° C. The results recorded by Preti (1908) as regards the insolubility of the hemolysin in salt solution and its resistance to boiling are at variance with those of other investigators, and, as will be shown in the following pages, are not in harmony with the results obtained by the present writer. Moreover, Preti's results can not be accepted as conclusive, owing to his failure properly to control his experiments. Alessandrin's attempt (1904) to associate the secretion of hemolysin with the cervical glands of the parasites is not sustained by Whipple (1909), who found the hemolysin in all parts of the worm.

It is interesting to observe that the different species of hookworms referred to in the foregoing summary have the common biological property of secreting a substance destructive to red blood cells. Inasmuch as hookworm disease is characterized by severe anemia, the presence of a blood-destroying substance in the parasites is highly significant.

In addition to the hemolytic substance which is present in hookworms, Loeb and his collaborators have shown that the hookworm parasitic in dogs (Ancylostoma caninum) also secretes a substance which inhibits coagulation of blood in vitro (Loeb and Smith, 1904; Loeb and Smith, 1906; Loeb and Fleisher, 1910). The results of experiments by these investigators with reference to the anticoagulins of hookworms may be summarized as follows: In A. caninum a substance is present which retards coagulation of blood in vitro. This substance which is present in the anterior part of the worm and practically absent in the posterior part is not destroyed but is markedly weakened by boiling for 15 minutes. The substance does not resemble hirudin, a toxic constituent of the leech, but appears to resemble cobra venom so far as its physiological properties are concerned. It is of interest to note in this connection that Lieffmann (1905), who rejects the view that the hookworm secretes a hemolysin, likewise rejects the view that this parasite secretes an anticoagulin, since he obtained positive results in but one out of three experiments which he performed. Lieffmann ascribes his positive results to substances from the intestine which may have adhered to the worms, namely, pancreatin and peptone. Loeb and Smith (1906) point out, however, that in view of the fact that they washed the worms carefully and that neither peptone nor pancreatin is known to inhibit coagulation of dog's blood in vitro, and further, in view of the fact that the posterior parts of the hookworms showed but a slight anticoagulating effect on dog blood and that extracts of ascarids and tape-worms from dogs did not retard the coagulation of dog blood, Lieffmann's contention can not be sustained.

The carefully controlled experiments of Loeb and his collaborators leave no room for doubt as to the presence of a hemotoxin in Ancylostoma caninum which inhibits the coagulation of dog blood. Loeb and Smith ascribe etiological significance to this toxin and believe that it has the power of causing small hemorrhages in regions of the intestine that have been lacerated by the worms.

The pathological rôle of the whipworm (Trichuris trichiura) parasitic in man is emphasized by Askanazy (1895), who states that this parasite feeds on blood, basing his assertion on the presence of iron pigment in the intestine of the worm demonstrable by the Berlin blue reaction. Askanazy assumed, of course, that the iron found in the worm is obtained from the hemoglobin of the host's blood. Schultze (1905) rejects Askanazy's interpretation and considers that the pigment in question is obtained from the host's intestine rather than from the blood.

Guiart (1908) presents conclusive evidence as regards the bloodsucking habit of Trichuris trichiura, since he found blood-engorged specimens in a human patient. Guiart's observation has been confirmed by a number of investigators, including Garin,
Seidelin, and Leon (Guiart, 1914). Guiart and Garin (1909) found that the presence of Trichuris eggs in the feces of human subjects is correlated with the presence of blood in the feces as shown by a positive Weber test.

As to the presence of hemotoxic secretions in whipworms, Whipple (1909), who experimented with extracts of these parasites, found that they contained a hemolytic substance destructive to the red blood cells of the dog and of man. Whipple states that the hemolysin left some samples of human red blood cells intact but was destructive to others. Garin (1913) performed similar experiments with Trichuris extracts and confirmed the presence of a hemolysin in these parasites. According to Garin, the whipworm hemolysin is thermostabile, being destroyed by 30 minutes' heating at 56° C. The inactivated hemolysin can not be reactivated by normal guinea-pig serum (complement), according to this investigator. Garin states, furthermore, that whereas he obtained positive results with human red blood corpuscles the results of experiments with the erythrocytes of rabbits and guinea pigs were doubtful.

A survey of the literature relating to the pathogenic rôle of Ascaris lumbricoides reveals the fact that this parasite may be responsible for anemia, which is sometimes mistaken for hookworm anemia or for pernicious anemia. The clinical reports of Demme (1891) have become a classic illustration of this fact. In brief, Demme found a child suffering from severe intestinal catarrh, with a high-grade pernicious anemia showing a red blood count of 2,450,000 and a hemoglobin content of 40 per cent. Two weeks after numerous worms (A. lumbricoides) had been expelled from the child's intestine the red blood corpuscle count rose to 4,200,000 and the hemoglobin content reached 70 per cent. In a second case of apparent pernicious anemia, which resulted in death and in which the erythrocytes had diminished to 1,650,000 per cubic millimeter, numerous ascarids were found on post-mortem examination which were apparently responsible for the death of the child. Kuttner (1865) found that in a girl aged 12 blood destruction occurred and that this was cured by expelling a number of ascarids. According to Filatoff (1897), Karaven cured a case of pernicious anemia in a child by expelling a number of ascarids from its intestine. François (1906), in the course of his investigations on anemia of miners, found many cases of severe anemia in which hookworms were not present but which showed numerous Ascaris eggs in the feces. A number of observations by different investigators on hogs and horses infested with ascarids and on man infested with A. lumbricoides bear out the fact that symptoms of anemia are frequently associated with such infestation.

As to the manner in which species of Ascaris cause anemia two views have been advanced, which are not mutually exclusive. Guiart (1899), who accepts the view that worms of this genus secrete a hemolysin, inclines strongly to the view that they also lacerate the mucosa, thus causing hemorrhages. In support of this view Guiart describes and figures Ascaris conocephala attached to the stomach of a dolphin, the head of the parasite being deeply embedded in the mucosa. Guiart refers to the observations of Leroux, who found lesions in the intestine of a human being infested with ascarids resembling lesions produced by ascarids on the mucosa of the dolphin. Friedberger and Fröhner (1895) also support this view and state that dogs that harbor numerous ascarids show on post-mortem examination of the intestine numerous round, dark spots, surrounded by an inflamed zone, due, in their opinion, to bites of the worms. According to Garin (1913), several observers, including Weinberg, have found ascarids attached to the mucosa. Garin admits, however, that despite the fact that he made numerous post-mortem examinations of human subjects infested with A. lumbricoides and of dogs and cats infested with ascarids, in the latter cases shortly after death, attached parasites were never observed by him. He confirms, however, the presence of reddish points surrounded by an ecchymotic area in the mucosa of the intestine of infested subjects, both human and animal. Thaler (1918) has recently reported a case of persistent intestinal hemorrhages in a human subject which did not
yield to symptomatic treatment and which was cured only after removing several ascarids.

The view that Ascaris secretions contain hemotoxins was first advanced by Schimmelpfennig (1902), who found that in the presence of the coelomic fluid of Ascaris equorum red blood corpuscles of the horse became crenated and were ultimately destroyed. Schimmelpfennig furthermore discovered oxyhemoglobin in the coelomic fluid of the parasite, a fact which led him to regard this worm as a bloodsucker. Weinberg (1907), Whipple (1909), and Alessandrini (1913) failed to observe any toxic effect of salt-solution extracts of species of Ascaris on red blood cells. Flury (1912), on the other hand, records the presence of strong hemolysins in the coelomic fluid of species of Ascaris. Flury ascribes the hemolytic action of Ascaris secretions to free fatty acids, of which oleic acid is the most active principle. In the course of his studies on the pharmacology of salt-solution extracts of worms of the genus Ascaris, Brinda (1914) found that injection of the extracts into guinea pigs brings about a reduction in the number of erythrocytes and a diminution in the hemoglobin content of the blood. Recently Shimamura and Fujii (1917), in the course of their investigations on "askaron," a toxic constituent of worms of the genus Ascaris, state that ether-soluble and alcohol-soluble fractions of Ascaris material contain a hemolytic agent. The present writer (Schwartz, 1919), in a preliminary paper on the hemolytic effects of Ascaris extracts, has briefly described the properties of the hemolysin.

A number of investigators have found, moreover, that the coelomic fluid of worms belonging to the genus Ascaris contains a substance that inhibits the coagulation of blood. Weil and Boyé (1910) found that as a result of injecting the fluid of Ascaris equorum into rabbits the blood of the latter when drawn remains uncoagulated for 20 minutes longer than blood from a normal rabbit. Experiments with rabbit blood and Ascaris fluid in vitro yielded negative results, according to these investigators. Leroy (1910) likewise observed that the blood of dogs which had been injected with the body fluid of A. equorum coagulated more slowly than blood from normal dogs. Flury (1912) observed that Ascaris fluid delayed the coagulation of dog blood and of human blood in vitro. That Loeb and Smith (1904) failed to observe anticoagulins in extracts of dog ascarids that are active in vitro has already been mentioned.

Worms belonging to the genus Strongylus (frequently referred to as Sclerostomum) are parasitic in the large intestine of horses. These nematodes attack the mucosa, to which they may be found adhering by means of their buccal capsule. In view of the fact that these parasites somewhat resemble hookworms in their attacks on the intestinal mucosa and in the effects which they produce on the host, Weinberg (1907) investigated their hemotoxic secretions primarily with a view of throwing light on the causes of anemia due to hookworms. This investigator found that physiological salt-solution extracts of freshly collected Strongylus material dissolves erythrocytes of horses, cattle, sheep, rabbits, and guinea pigs. The parasites secrete, therefore, a nonspecific hemolysin. Weinberg determined that the hemolysin is thermostable, resisting heat at a temperature of 115° to 120° C. for 15 to 20 minutes. In addition to the hemolysin, Weinberg found that these parasites secrete a substance which inhibits the coagulation of horse blood in vitro. He also found that salt-solution extracts of worms of the genus Strongylus contain a substance which when brought in contact with the blood serum of the horse causes the formation of a precipitate. The precipitin, too, is nonspecific in its action, since it was found by Weinberg that it produces a precipitate when added to rabbit-blood serum.

Bondouy (1908, 1910) studied the chemical composition of worms belonging to the genus Strongylus, with special reference to their hemolytic constituents, and confirmed in the main the results obtained by Weinberg as regards the presence of a soluble hemolysin in these parasites. The new facts discovered by Bondouy may be briefly summarized as follows: The parasite contains soaps and free fatty acids which exert a destructive effect on red blood cells in vitro. Bondouy states, however, that the
presence of these substances in the parasite is due to its blood sucking habit, basing his assertion on the fact that blood serum contains neutral fats, fatty acids, and soaps. This writer found a lipolytic enzym in worms of the genus Strongylus which apparently converts the storage fat into fatty acid. It is of interest to note also that Bondouy found neither lecithin nor cholesterin in the parasite. Lecithin, as is known, has the property of activating certain hemolytic agents, namely, snake venoms, whereas cholesterin inhibits hemolysis of blood by active hemolysins. Contrary to Weinberg's experience (Weinberg, 1907), Bondouy found that Strongylus hemolysin is soluble in alcohol. From the alcohol-soluble fraction of the parasite this writer isolated an extremely active hemolysin which he identified as an alkaloid. He also found a ptoin in the parasites which exhibited hemolytic properties.

Brumpt and Joyeux (quoted by Brumpt, 1910) found that a watery extract of the stomach worm of sheep (Haemonchus contortus) produced a slight hemolytic effect after 2.4 hours and a total hemolysis after 12 hours. Cuillé, Marotel, and Panisset (1911) state that extracts of sheep strongyles (species of which apparently several were involved, not given) did not exert any effect on sheep red blood corpuscles from either healthy or sick animals. These writers also state that extracts of these parasites contained hemoglobin.

According to Garin (1913) Graphidium strigosum and Trichostrongylus retortaeformis, nematodes parasitic in the stomachs of hares and rabbits, secrete hemolysins. With reference to the hemolysin of G. strigosum, Garin found that it is secreted by the living worm in vitro. He found, furthermore, that the hemolysin is apparently a complex substance and acts on the blood not directly but in combination with complement. Heating at 55\(^{o}\) C. for 30 minutes does not destroy but merely inactivates the hemolysin, which may be reactivated by normal serum, according to this investigator. In view of the limited number of experiments which Garin performed, his conclusions can be accepted with reservation. The work requires confirmation. As for the hemolysin from T. retortaeformis, Garin found it to be far less potent than that of G. strigosum. He also states that the hemolysins from the two species have far greater affinity for the blood cells of rabbits than for those of other species of animals and are therefore relatively specific.

Yagi (1910) found that salt-solution extracts of the blood fluke, Schistosoma japonicum, are hemolytic to erythrocytes of cattle, sheep, and rabbits. He found, furthermore, that this hemolysin is soluble in ether and concluded that it is probably a fatty acid. Yoshimura (1913) experimented with salt-solution extracts of the same species and found them to be destructive to rabbit erythrocytes. Human blood cells, according to this writer, are refractory to these extracts. Yoshimura also experimented with ether extracts, which he found destructive to rabbit red blood corpuscles and to a lesser extent destructive to human red blood corpuscles.

According to Guerrini (1908), Fasciola hepatica secretes a hemolysin which is absorbed by the host and is demonstrable in the blood serum of the latter.

Alessandrini (1913) records the results of experiments with extracts of Macracanthorhynchus hirudinaceus, the thorn-headed worm of the hog. He tested the body fluid and extracts of various parts of the worm and found them to be destructive to the red blood cells of swine, cattle, and sheep. Alessandrini states that the hemolysin from M. hirudinaceus is a colloidal substance insoluble in alcohol, soluble in water, and highly sensitive to heat, since a temperature of 40\(^{o}\) C. diminished its potency and a temperature of 55\(^{o}\) destroyed it entirely.

Although the larvae of species of Gastrophilus which occur in the stomach of the horse are in a zoological sense not parasitic worms, the results of a study of their toxic secretions may be included in this review because these larval parasites are biologically more closely related to parasitic worms than they are to free-living insect larvae. At any rate their secretions may be absorbed by the host and give rise to disturbances.

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1 No details are given as to kind and quantity of blood corpuscles used.
similar to those produced by the secretions of helminths. Weinberg (1908) investigated the hemotoxic properties of the fluid of these parasites and obtained the following results: Extracts of the intestine and of the red cells of the fatty bodies of the larvae contain a soluble hemolysin, nonspecific in its action and susceptible to heating for \( \frac{1}{2} \) hour at 56° C., which does not destroy it but merely weakens its potency. Weinberg found, moreover, that these extracts have an inhibiting action on the coagulation of the blood of several species of animals.

**SUMMARY**

Summarizing the results of hitherto recorded investigations on hemotoxins from parasitic worms, it may be stated that while there is more or less contradictory evidence in the literature the following facts have apparently been established:

1. Certain parasitic worms secrete substances that affect the blood of their host deleteriously. These substances, which may be designated as hemotoxins, are in general nonspecific in the sense that they are also active toward blood of animals other than their normal host.

2. *Diphyllobothrium latum*, a tapeworm which is known to cause severe anemia, contains a hemolytic agent. It appears questionable that this agent is oleic acid, as claimed by Faust.

3. Concerning hemolysins in cestodes other than *Diphyllobothrium latum* no definite conclusions can be drawn from the literature on the subject, but that hemolysins are present in several species appears probable.


5. Hookworms (Ancylostoma and Necator) secrete a hemolysin and an anticoagulin.

6. Whipworms (*Trichuris trichiura*) apparently secrete a hemolysin.

7. Worms belonging to the genus Ascaris contain a hemolysin which is closely bound to the tissues of the worms and is therefore but slightly soluble in water, which fact accounts for the negative results obtained by certain investigators. These parasites also appear to secrete a feeble anticoagulin.

8. Worms of the genus Strongylus secrete a hemolysin and an anticoagulin. The hemolytic principle of these parasites is apparently an alkaloid, although other substances found in them show hemolytic power.

9. *Haemonchus contortus* apparently secretes a weak hemolysin.

10. Extracts of *Macracanthorhynchus hirudinaceus* are apparently destructive to erythrocytes.

11. Hemolytic and anticoagulating properties are found in extracts of the larvae of species of Gastrophilus.

12. Hemolytic substances from parasites are soluble in alcohol\(^1\) and ether, thus resembling lipoids.

13. With respect to their resistance to heat, hemolysins from animal parasites vary, but in general they are thermostable.

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\(^1\) According to Alessandrini the hemolysin from *Macracanthorhynchus hirudinaceus* is insoluble in alcohol.
Owing to the fact that the direct abstraction of blood by parasites appears to be inadequate as an explanation of the causes of anemia in parasitic diseases, and in view of the fact that in tapeworm infections which are accompanied by anemia due entirely to the presence of the parasites the direct abstraction theory is inapplicable, the view that hemolysins from parasites are of etiological significance in parasitic diseases appeared to be entirely justified.

III. TECHNIC

Unless otherwise indicated, the experiments described in the following pages were performed with washed red blood cells. In most cases the blood was defibrinated, filtered through gauze, centrifuged to remove the serum, and washed in physiological salt solution at least three times to free it from traces of serum. In a few cases a somewhat different procedure was followed. The blood was collected in a 2 per cent solution of sodium citrate or in physiological salt solution containing 1 per cent sodium citrate. The removal of the serum and subsequent washing in physiological salt solution were carried out as in the case of defibrinated blood. Unless otherwise stated, a 5 per cent suspension of corpuscles, made by suspending 1 part of washed red blood corpuscles in 19 parts of physiological salt solution, was used.

Blood serum used in these experiments was obtained as follows: In the case of rabbits blood was obtained by severing the marginal ear vein, and in the case of the larger domestic animals it was obtained at an abattoir from animals that were being bled and was allowed to drop into a sterile centrifuge tube. The tube containing the blood was allowed to remain at room temperature for a few hours. By means of a sterile platinum wire the clot was loosened from the sides of the tube to which it adhered and the tube was then centrifuged. The clear serum was pipetted off, and if the serum was to be kept for more than three days sufficient phenol was added to give a phenol content of 0.25 to 0.5 per cent; otherwise no preservative was added.

Extracts of parasites were made from fresh material and from dried material. In both cases the living specimens were obtained shortly after they had been removed from the host. Certain writers who deny the presence of toxic substances in parasitic worms base their objection to the evidence in favor of the view that parasitic worms secrete toxic substances on the grounds that extracts are frequently made from parasites that are obtained as a result of anthelmintic medication and that the toxicity may be due to traces of anthelmintic which adhere to the surface of the parasite or to secondary degeneration products of dead worms. The present writer has been careful to use fresh specimens in order to avoid complications of the sort just mentioned. It should also be stated that the parasites obtained from the intestines and other organs were washed in physiological salt solution and were transferred three or
four times in succession to fresh salt solution. In this manner the surface of the worms was freed from adhering intestinal material. In the case of salt-solution extracts that were allowed to remain at room temperature or in an incubator for several hours or for a few days, a preservative, usually a few drops of chloroform, was added to the extract to inhibit bacterial growth.

Specimens were dried as follows: After having been washed a number of times in physiological salt solution, the surface of the worms was dried with filter paper. The specimens were then placed in a single layer in a glass dish and allowed to dry either at room temperature in an incubator or in vacuum over sulphuric acid. Small worms dry in a few hours, even at room temperature, and become sufficiently crisp to be pulverized. Larger specimens dry more slowly and are usually crisp in about 48 hours. The dried material was triturated in a mortar and stored in bottles, usually in a dark place.

Special points in technic are covered in connection with the different series of experiments and are not taken up in this connection.

As used in this paper, the terms physiological salt solution and salt solution refer to an 0.85 per cent solution of sodium chlorid in distilled water.

Controls on all samples of blood corpuscles used in the experimental work described in the following pages were maintained in connection with each experiment or series of experiments.

IV. EXPERIMENTS WITH HEMOLYTIC EXTRACTS OF ASCARIS LUMBRICOIDES

I. METHOD OF OBTAINING FLUID FROM WORMS

Body fluids and extracts were obtained from specimens of Ascaris lumbricoides from swine. A supply of these parasites is available in abattoirs during all seasons of the year.

The fluid which is present in the body of the worms was usually obtained by cutting off the posterior end of medium-sized to large-sized specimens and allowing the pinkish liquid to drop into a test tube. Fluid obtained in this manner does not keep well and is available only for immediate use. Allowed to stand, even at a low temperature, the body fluid thus collected undergoes bacterial decomposition in about 24 to 36 hours. Weinberg and Julien (1911) describe a method of collecting Ascaris body fluid under aseptic precautions. Briefly, the method consists in drying the worms with filter paper, holding the ends of each specimen and passing the middle region of the worm through the flame of a Bunsen burner until the cuticle bursts. The first two or three drops of fluid which ooze out are discarded and the remaining fluid is allowed to drop into a sterile tube. This procedure was tested by the present writer with inconstant results so far as the keeping qualities of
the fluid were concerned. In some cases sterile fluid was obtained in this manner, but more often the fluid became contaminated. The contamination was extraneous and not inherent in the body fluid of the worms, since a number of experiments performed by the writer showed quite conclusively that the intact body fluid of Ascaris is sterile.

In the course of the experimental work described in this paper specimens were kept alive in vitro for a few days. This necessitated information as to the conditions that are favorable to the survival of the parasites outside of the host. The customary procedure of keeping parasitic worms at a low temperature is not applicable to Ascaris lumbricoides when considerable periods, generally in excess of 24 hours, are involved. Incubator temperatures (37.5°C.) are more favorable than refrigerator temperatures, but so far as longevity of the worms outside the host is concerned, a temperature ranging from above 25° to 32° was found to be the most favorable. The worms were kept in shallow dishes and in beakers, and sufficient salt solution was added to cover the worms. Fluid from worms that had thus been subjected to starvation was obtained in the same manner as fluid from fresh worms.

2. EXPERIMENTS WITH THE BODY FLUID OF ASCARIS LUMBRICOIDES

In nematodes the space between the body wall and the gut wall is filled with a fluid which in the case of such large-sized worms as those of the genus Ascaris is available in quantities sufficient for investigation. According to Flury the body fluid of Ascaris equorum consists largely of water (95 per cent). Other substances present in this fluid, according to the same investigator, are albumin, globulin, and other proteins, soaps, free fatty acids, various katabolic products of proteins, purin bases, and their derivatives, sodium chloride and other inorganic substances, as well as digestive and oxidizing enzymes. Flury found that the body fluid of A. lumbricoides is physically and chemically indistinguishable from that of A. equorum.

The fact that the body fluid of Ascaris lumbricoides, which in fresh specimens has a bright pinkish color, contains oxyhemoglobin is of great significance. The presence of oxyhemoglobin in the worms may be readily demonstrated by means of the spectroscope. Schimmelpfennig (1902) appears to have been the first investigator to note this fact, on the basis of which he ascribed to worms of the genus Ascaris the rôle of blood-suckers. This investigator also states that worms belonging to this genus liberate their oxyhemoglobin content into the physiological salt solution in which they are kept alive in vitro. The presence of iron granules in the gut wall of ascarids was affirmed by Askanazy (1896), who bases his view on positive Berlin blue tests, the inference being that the pigment in question is obtained from the blood of the host. Flury (1912) refers to the presence of hemoglobin in ascarids and states that he observed it in worms which had been kept for two weeks in an incubator. Flury
inclines to the view that oxyhemoglobin is a normal constituent of these worms. Dobernecker (1912) records the presence of oxyhemoglobin in ascarids, which he determined by means of the spectroscope. Fauré-Fremiet (1913) expresses the view that the oxyhemoglobin present in the intestine of worms belonging to the genus Ascaris is obtained from the blood of the host and that the iron pigment in the intestinal cells is derived from disintegration products of hemoglobin. Galli-Valerio (1915) affirms the presence of blood in ascarids and states that the body fluid of a female ascarid gave a positive benzidin test for blood. The present writer (Schwartz, 1919) found that Ascaris lumbricoides loses its oxyhemoglobin when kept in vitro for a number of days and that coincident with the loss of this substance the worms become sluggish and die. Magath (1919) has made a similar observation in the case of another nematode (Camallanus americanus) which contains a “reddish fluid.” Magath also notes the presence of pigment granules in the gut wall of this worm.

It has already been stated in another section of this paper that Schimmelmannig (1902) and Flury (1912) found that the body fluid of worms belonging to the genus Ascaris is destructive to red blood cells. Following are the observations and experiments of the present writer on this question.

Fluid collected from fresh specimens of Ascaris lumbricoides within 24 hours after removing the parasites from the host is not hemolytic. Such fluid was tested on the washed erythrocytes of cattle, sheep, hog, rabbit, and guinea pig without producing any appreciable dissolving action. In one case it was found that fluid which had been kept in a refrigerator for three days was destructive to sheep erythrocytes, but a repetition of this experiment with fluid from another lot of worms yielded negative results. Fluid collected under aseptic precautions and kept in a refrigerator for two or three days failed to hemolyze red blood corpuscles.

On the other hand, fluid from worms which had been kept alive in vitro for a number of days was found to be hemolytic. In one case worms were kept alive in a physiological salt solution for eight days at a temperature of 32° to 33° C. At the end of this period fluid was obtained from the worms and tested on the washed red blood cells of the hog, with positive results. A repetition of this experiment on a different sample of washed erythrocytes from the hog likewise yielded positive results. In another case worms which were kept alive for six days yielded a fluid which was destructive to washed sheep corpuscles. Fluid from another lot of worms which had been kept in the laboratory for four days was but slightly although quite unmistakably hemolytic to sheep blood corpuscles. A portion of this fluid was boiled and the clear liquid after being separated from the coagulum was still hemolytic. Fluid from
worms which has been kept alive for eight days was strongly hemolytic to washed sheep blood corpuscles.

In the course of these experiments it was observed that whereas fresh specimens of *Ascaris lumbricoides* from swine are pink in appearance they become white as they are kept in the laboratory. Spectroscopic examination of the fluid showed that the pink appearance is correlated with the presence of oxyhemoglobin and the white appearance is correlated with the absence of that substance. In other words, worms kept in vitro lose their oxyhemoglobin, a fact which appears to indicate that this substance is not a constant constituent of the worm but that it is obtained from the host, the supply evidently being renewed from time to time. Inasmuch as Schimmelpfennig (1902) states that the oxyhemoglobin is eliminated in vitro, the present writer made spectroscopic examinations of physiological salt solution in which ascarids had been kept alive for 24 hours or longer, and found that such solutions did not show the oxyhemoglobin spectrum. Tests for iron in such salt solutions showed but slight traces of this substance. That these traces were excretion products of the parasite was shown by the fact that a quantity of salt solution from the same supply which was added to the beakers in which the worms were kept gave negative results. It may be concluded, therefore, that when removed from the host and kept in a physiological salt solution living ascarids lose their oxyhemoglobin content not by excreting it as such but probably by breaking it down into simpler substances and storing the iron in their tissues. The fact that ascarids are rich in iron and that this substance enters in considerable quantities into the composition of the eggs (Schimmelpfennig, 1902) is decidedly significant in this connection.

On the basis of certain experiments Flury (1912) states that salt solutions in which living ascarids have been kept for 24 hours have absorbed the hemolysin which the parasites excrete. The observations of the present writer on this point do not bear out Flury's view, as the following experiments will show.

A number of swine ascarids were kept in a beaker for 24 hours in a quantity of physiological salt solution sufficient to cover the worms. Ten cc. of this salt solution produced no dissolving effect on 1 cc. of a 5 per cent suspension of guinea-pig red blood corpuscles. A similar experiment was performed with a different lot of worms, the salt solution in this case being tested on washed hog erythrocytes, with negative results. Negative results on sheep erythrocytes were also obtained with salt solution in which another lot of worms had been kept for 24 hours. In a similar way negative results were obtained on several other occasions with salt solution in which living ascarids had remained from 18 to 36 hours.

In the experiments mentioned above the parasites were examined and found to be still alive before the salt solution was tested as to its hemolytic
property. In another series of experiments in which some of the worms were found to be dead it was observed that the salt solution in which they had been kept was destructive to red blood corpuscles. That the hemolytic effects of salt solution in which dead ascarids had been kept was independent of bacteria was shown by the fact that the salt solution was free from putrefactive odors associated with decay, due to the precautions which were taken to free the parasites from bacteria by immersing them in 2 per cent formalin and washing them first in water and then in salt solution before subjecting them to these experiments. In one experiment which was conducted under strictly aseptic precautions the worms were thoroughly washed in running water, in formalin, and in sterile salt solution in the order indicated and then placed in sterile flasks containing an 0.85 per cent solution of sodium chlorid. These flasks were placed in an incubator at 37° C. for several days. The worms died, but the fluid showed no cloudiness. Transfers of portions of this fluid to culture media (nutrient broth and agar) failed to produce bacterial growth despite the fact that the tubes containing the media were kept in the incubator for a week. The sterile salt solution in which the ascarids died was hemolytic to washed sheep corpuscles.

These facts appear to indicate that Ascaris hemolysin is closely bound to the cells of the parasites and becomes dissociated from them rather easily after death of the worms, a view which is in harmony with the observation of Tallqvist (1907) with reference to the hemolysin from Diphyllobothrium latum. The fact that the body fluid of worms which have been kept in vitro for a number of days becomes hemolytic is entirely in harmony with that view, since, under conditions of starvation, autolysis of the tissues of the parasites undoubtedly takes place, especially after the storage products, largely glycogen, are consumed.

3. EXPERIMENTS ON THE POSSIBLE PRESENCE OF COMPLEMENT IN THE BODY FLUIDS OF ASCARIS LUMBRICOIDES

Experiments with body fluid from fresh specimens of Ascaris lumbricoides were performed with a view to determining whether it contains a substance capable of activating a hemolytic system. As is well known, washed red blood corpuscles to which a specific inactivated antiserum is added will not hemolyze unless a certain quantity of normal fresh blood serum is added. The substance in the normal blood serum which in itself has no hemolytic power but which activates inactivated antiserum is known as alexin or complement. Comparatively little is known of this body except that it is a normal constituent of blood serum, that it deteriorates rapidly in vitro, and that it is destroyed by heating at 56° C. for 30 minutes. According to Noguchi (1907), soluble soaps to which

1 Schulte and Krummacher (1913) have shown that starving ascarids do not consume their fat content and have confirmed Weinland’s views with reference to the rôle of glycogen in the metabolism of the worms in vitro.
inactivated serum is added act as complement; in other words, a mixture of inactivated serum and soap can activate a hemolytic system (washed red blood corpuscles plus specific antiserum).

The present writer endeavored to answer the following questions: Is the fresh body fluid of *Ascaris lumbricoides*, which, as has already been shown, has no hemolytic power, capable of activating a hemolytic system? In other words, does it contain complement? Second, can a combination of inactivated serum and an alcoholic extract of body substance of *A. lumbricoides* from which the ether-soluble fraction has been removed, and which contains whatever soluble soaps the parasite has, activate a hemolytic system? The answers to these questions will be found in the results of the following experiments.

One cc. of washed sheep red blood corpuscles was mixed with a unit of specific inactivated antiserum (amboceptor) determined by previous titration. To one tube containing this mixture a certain quantity of fresh guinea-pig serum (complement) was added, sufficient to activate the amboceptor—that is, to cause it to combine with the blood corpuscles and to produce hemolysis. The quantity of complement necessary to activate the hemolytic system was determined by previous titration. Hemolysis was produced in 30 minutes at 37° C. To a series of 10 tubes containing the mixture of amboceptor and sheep red blood corpuscles various quantities of body fluid collected from living swine ascarids under aseptic precautions shortly after the worms had been removed from their hosts were added. The quantities of fluid added to these tubes ranged from 0.1 cc. to 10 cc. These tubes were shaken and incubated at 37° C. for one hour. No hemolysis was observed in any tube. The tubes were then put in a refrigerator for 20 hours longer, but the blood corpuscles remained intact. It should be stated in this connection that the body fluid in question was free from bacteria, since a portion of it was thoroughly mixed with melted agar which was plated and incubated. The plates remained sterile. Ascaris fluid lacks, therefore, a substance (complement) which is capable of activating a hemolytic system.

As to the combination of inactivated normal serum with an alcoholic extract of *Ascaris lumbricoides*, the following experiment was performed: Dried ascarids were powdered, extracted in warm alcohol, and the alcoholic extract after evaporating the alcohol was washed with ether. The ether, as is known, removes neutral fats, fatty acids, lecithin, choles terin, and other lipoids. The ether-insoluble substance was then dissolved in salt solution and combined with normal guinea-pig serum that had been heated to 51° C. to determine whether this combination can act as complement, that is, whether it can activate a hemolytic system. To one

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1 The presence of soaps in ascarids is affirmed by Flury (1912).
2 According to Noguchi, similar chemical fractions of mammalian tissues combined with inactivated normal serum act as complement.
tube containing 1 cc. of a mixture of washed sheep red blood corpuscles and specific antiserum in the proper proportion as determined by previous titration, one unit of normal guinea-pig serum (complement) was added. (The unit of complement was determined by titration.) Hemolysis resulted. To a second tube containing a mixture of washed sheep red blood cells and specific antiserum one unit of inactivated complement (heated to 51°) was added. No hemolysis resulted. To a series of tubes containing washed sheep red blood corpuscles and specific antiserum various combinations of inactivated guinea-pig complement and alcoholic extract of *A. lumbricoides* were added. No hemolysis was produced in any of these tubes. It is evident, therefore, that *A. lumbricoides* not only lacks complement but that an alcoholic extract of the worm freed from all ether-soluble substances combined with inactivated normal serum can not act as complement.

In this connection it is of interest to note that Holland (1919) found that the blood of insects lacks complement and that this substance is also absent from the blood of mollusks. Cantacuzene (1919) examined the fluids of a number of invertebrates as well as of tunicates but failed to find complement. He succeeded, however, in producing complement in a crab (a species of *Eupagurus*) by artificial immunization with sheep red blood corpuscles.

Summarizing, *Ascaris lumbricoides* in common with other invertebrates lacks complement, a substance that is known to play an important rôle in the immunity processes of higher vertebrates. That *A. lumbricoides* and other internal parasites which live in parts of the body where bacteria are more or less abundant protect themselves against bacterial invasion is probable. The intestine of *A. lumbricoides* contains bacteria, as has been recorded by several investigators. The present writer found bacteria in the intestine, but the body fluid of fresh ascarids when collected under aseptic precautions was found to be sterile. That the body fluid and tissue extracts of ascarids and of other internal parasites contain bactericidal substances has been affirmed by a number of writers (Alessandrini, 1913).

4. **Experiments with Extracts of Entire Worms**

It has already been stated that Weinberg (1907), Whipple (1909), and Alessandrini (1913) failed to find hemolysins in salt-solution extracts of ascarids. Garin (1913) records the results of 10 experiments with extract of worms of the genus Belascaris, of which 8 yielded negative results and 2 yielded positive results on dog-blood corpuscles. These investigators experimented with extracts of fresh specimens made by macerating the worm material in physiological salt solutions. The present writer found that as a result of extracting *Ascaris lumbricoides* material by macerating fresh worm substance in salt solutions the hemolysin is seldom liberated.
from the tissues of worms. Better results were obtained by grinding up fresh worm material with sand and shaking the mixture of worm fragments and sand for a number of hours, followed by extraction in an incubator for a number of days. This procedure necessitated the addition of a preservative to the extract in order to prevent bacterial contamination. In experiments in which this procedure was followed, sufficient carbolic acid was added to make a 0.25 per cent solution; and in hemolytic tests controls involving the use of salt solution containing a similar quantity of carbolic acid were included. Following the procedure described above an extract of fresh worm material was made as follows: A few pieces (10 gm. by weight) of worm material from a number of different specimens were ground up with sand and suspended in 100 cc. of physiological salt solution containing 0.25 per cent of phenol. The mixture was shaken for a few hours in a shaking machine and then incubated, usually for three days, at 37°C. The extract was then filtered and a clear filtrate tested on various samples of red blood corpuscles as follows.

The filtrate was tested on washed erythrocytes of a number of cattle, sheep, hogs, rabbits, guinea pigs, and rats, with positive results. In most experiments it was found that 0.4 cc. of the extract hemolyzed 1 cc. of a 5 per cent suspension of red blood corpuscles. In a number of tests 0.2 cc. of the extract hemolyzed 1 cc. of the suspension of corpuscles. As a control on the phenol which was added as a preservative, 0.5 cc. and 1 cc. of a salt solution containing ½ per cent of phenol was tested on each sample of blood corpuscles used in the hemolytic tests, with negative results. Tests to determine whether normal serum contains antibodies were nearly always positive. From 0.2 to 0.5 cc. of serum was sufficient to inhibit hemolysis of 1 cc. of corpuscle suspension by from 0.2 to 0.4 cc. of the extract. Sometimes 0.1 cc. of serum brought about the same results.

That the activity of the hemolysis is independent of the acidity of the solution was shown by the fact that as a result of neutralizing the extract its activity was not destroyed. Furthermore, the hemolytic potency of the extract was not due to secondary degeneration products associated with acid production, because the hemolytic power of the extracts remained intact for a long period (several months), during which it was tested from time to time against different species of corpuscles. Moreover, filtrates of extracts of worms that were prepared by thoroughly triturating the specimens and adding a few drops of chloroform to inhibit bacterial growth during the few hours that the extracts were kept in a refrigerator were found to be hemolytic. An example of the results of experiments with salt-solution extracts of *Ascaris lumbricoides* on red blood cells is given in Table I, in which a few experiments are summarized.
Table I.—Effect of salt-solution extract of Ascaris lumbricoides on red blood corpuscles

<table>
<thead>
<tr>
<th>Kind of erythrocytes, b</th>
<th>Quantity of extract, c</th>
<th>Results after two hours at 37° C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig</td>
<td>0.1 cc.</td>
<td>—</td>
</tr>
<tr>
<td>Do</td>
<td>0.2 cc.</td>
<td>—</td>
</tr>
<tr>
<td>Do</td>
<td>Salt solution</td>
<td>—</td>
</tr>
<tr>
<td>Do</td>
<td>0.1 cc. (boiled)</td>
<td>—</td>
</tr>
<tr>
<td>Rat e</td>
<td>0.1 cc.</td>
<td>+</td>
</tr>
<tr>
<td>Do</td>
<td>0.2 cc.</td>
<td>++</td>
</tr>
<tr>
<td>Do</td>
<td>0.3 cc.</td>
<td>+++</td>
</tr>
<tr>
<td>Hog f</td>
<td>Salt solution d</td>
<td>—</td>
</tr>
<tr>
<td>Do</td>
<td>0.2 cc.</td>
<td>+</td>
</tr>
<tr>
<td>Do</td>
<td>0.4 cc.</td>
<td>+++</td>
</tr>
<tr>
<td>Cattle f</td>
<td>Salt solution d</td>
<td>—</td>
</tr>
<tr>
<td>Do</td>
<td>0.4 cc.</td>
<td>+</td>
</tr>
<tr>
<td>Do</td>
<td>Salt solution d</td>
<td>+++</td>
</tr>
</tbody>
</table>

a — indicates total absence of hemolysis. + indicates slight hemolysis. ++ indicates marked but incomplete hemolysis. +++ indicates complete hemolysis.

b One cc. of a 5 per cent suspension of defibrinated blood washed three times in physiological salt solution was used in experiments.

c The extract used in these experiments was made by suspending 10 gm. of fresh worm material in 100 cc. of 0.85 per cent NaCl.

d Two controls—0.5 cc. and 1 cc. of salt solution containing 0.5 per cent phenol were tested on 1 cc. of the suspension of corpuscles.

e Pooled blood from six rats.
f Four samples of corpuscles were tested.

5. EXPERIMENTS WITH ASCARIS LUMBRICOIDES POWDER

The hemolytic principle of Ascaris lumbricoides may be preserved by drying the parasites. Specimens collected at a local abattoir were washed in salt solution to remove adhering intestinal débris, dried superficially with filter paper, and then placed in vacuum over sulphuric acid. When the specimens were sufficiently crisp they were powdered in a mortar and stored for future use. Ascaris lumbricoides powder when added to a suspension of washed blood cells of cattle, sheep, swine, etc., produces rapid hemolysis. As in the case of extracts of the parasite, the hemolytic action is inhibited by normal serum. The hemolytic substance may be more easily obtained from dried than from fresh ascarids by extracting the worm material in physiological salt solution. This is no doubt due to the fact that the dried material can be readily crushed and the hemolytic substance which, as has already been indicated, is rather closely bound to the parasite, may be more readily liberated. The following experiments performed by the writer illustrate this point: Several swine ascarids were broken up into small fragments but were not powdered in a mortar. A portion of this material was extracted in salt solution for a few hours and filtered. The filtrate was tested on washed sheep corpuscles with negative results. The remaining portion of dried worm material was thoroughly ground
in a mortar, extracted in physiological salt solution, filtered, and the filtrate tested on sheep corpuscles. The results in this case were positive.

A number of experiments were made with salt-solution extracts of powdered *Ascaris lumbricoides*. Rabbit and sheep corpuscles were used in nearly all experiments with these extracts. The results of these experiments were positive when the extracts were made from thoroughly powdered material; otherwise the extracts were only slightly hemolytic.

Extracts of powdered material of *Ascaris lumbricoides* were usually prepared as follows: A definite quantity of powder was added to a definite volume of physiological salt solution in a flask, the latter was shaken thoroughly, and the material was extracted for a few hours to two days in a refrigerator without the addition of any preservative, or extracted in an incubator, in which case a few drops of chloroform were added. The mixtures were then filtered, and in cases in which chloroform had been added the filtrate was left in an open receptacle in order to get rid of the chloroform by evaporation. The salt-solution filtrates were then tested as to their hemolytic power.

An example of results of these experiments is given in Table II, in which a number of tests are summarized.

**Table II.—Effects of salt-solution extracts of powdered *Ascaris lumbricoides* on red blood corpuscles**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Kind of erythrocytes</th>
<th>Quantity of extract</th>
<th>Results after 2 hours at 37° C.</th>
<th>Results after 20 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rabbit</td>
<td>5 drops</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>do</td>
<td>8 drops</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>do</td>
<td>8 drops (boiled)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>4</td>
<td>do</td>
<td>10 drops (boiled)</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>5</td>
<td>do</td>
<td>10 drops (heated at 60° C., 30 minutes)</td>
<td>+++</td>
<td>−</td>
</tr>
<tr>
<td>6</td>
<td>do</td>
<td>10 drops salt solution</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>7</td>
<td>Sheep</td>
<td>8 drops</td>
<td>−</td>
<td>+++</td>
</tr>
<tr>
<td>8</td>
<td>do</td>
<td>10 drops</td>
<td>+++</td>
<td>−</td>
</tr>
<tr>
<td>9</td>
<td>do</td>
<td>10 drops salt solution</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>10</td>
<td>do</td>
<td>8 drops</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>11</td>
<td>do</td>
<td>10 drops</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>12</td>
<td>do</td>
<td>10 drops salt solution</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

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*a* indicates negative results. + indicates slight hemolysis. + + indicates marked but incomplete hemolysis. - + + indicates complete hemolysis.

*b* Five drops of a 5 per cent suspension of washed rabbit erythrocytes and a 3 per cent suspension of washed sheep erythrocytes were used in these experiments.

*c* In experiments 1 to 8, inclusive, the following extract was used: 0.85 gm. of powder were suspended in 85 cc. of salt solution and extracted in an incubator for 24 hours. In experiments 10 to 12 the extraction was made as follows: 1 gm. of powder was extracted in 10 cc. of salt solution in a refrigerator.

*d* After remaining in an incubator for 2 hours the tubes containing the corpuscles and extracts were in some instances transferred to a refrigerator (8° C.) where they were kept for 18 hours longer before the final reading was taken.
6. EXPERIMENTS WITH EXTRACTS OF DIFFERENT ORGANS OF ASCARIS LUMBRICOIDES

It has already been stated that the body fluid of fresh specimens of *Ascaris lumbricoides* is not hemolytic and that this fluid acquires hemolytic properties as the parasites are kept in vitro. Extracts of entire worms, on the other hand, were found to contain a hemolytic substance which is apparently firmly bound to the tissues of the parasite. These facts appear to indicate that the hemolytic substance is liberated in rather small quantities and that it ultimately finds its way into the body fluid. That the liberation of hemolysin from the tissues and cells of the parasite is associated with metabolic processes of the worms is advanced as a plausible explanation of the facts. In the host animal the body fluid of the worm contains blood and blood products by which the hemolysin is apparently neutralized. In vitro, on the other hand, the blood elements disappear, as judged by the disappearance of oxyhemoglobin; and meanwhile fresh hemolysin which has found its way into the fluid remains unbound.

The question as to which morphological elements of *Ascaris lumbricoides* secrete the hemolytic substance or substances is interesting. A number of specimens of the parasite were therefore dissected and the intestine, reproductive organs, and body wall were separated into different lots. Physiological salt-solution extracts from each lot were tested on hog blood, and in a few cases on sheep blood.

In one series of experiments it was found that the extracts of the intestine were strongly hemolytic, whereas extracts of the body wall showed no hemolytic effects. Extracts of the reproductive organs were moderately hemolytic. In a second series of experiments extracts of the intestine were found to be very markedly hemolytic, whereas extracts of the body wall and reproductive organs showed weak hemolytic power.

In another series of experiments a number of worms were dissected, and the body wall, reproductive organs, and chyle intestine were separated into different lots. Each lot was washed in physiological salt solution and dried with filter paper. The material in each lot was then put in an incubator at 40° C. and allowed to remain there for 24 hours. Pulverized material from each lot was then suspended in physiological salt solution and tested on washed sheep corpuscles. Extract of the intestine produced rapid hemolysis at 37° (in about 1 hour), whereas extract of body wall of approximately the same strength as that of the intestine produced no hemolysis even after 3 hours at 37° followed by 18 hours in a refrigerator. Extract of the reproductive organs produced no hemolysis after 3 hours at 37° but after an additional period of 18 hours at 8° a slight indication of hemolysis was observed.
It may be concluded, therefore, that the hemolytic agent of *Ascaris lumbricoides* is primarily a secretory product of the intestine and that part of this substance finds its way into the body fluid where it is apparently neutralized by blood elements that are obtained from the host.

7. EXPERIMENTS WITH DIFFERENT CHEMICAL FRACTIONS OF *ASCARIS LUMBRICOIDES*

In contrast to the comparatively slight solubility of the hemolytic substance of *Ascaris lumbricoides* in physiological salt solution is its ready solubility in lipoid solvents, especially in alcohol. Equal quantities of powder were suspended in 5 cc. each of physiological salt solution, 95 per cent alcohol, ether, and acetone for 48 hours. The filtrates were evaporated and redissolved in 5 cc. of physiological salt solution. These extracts were then tested on a 5 per cent suspension of washed rabbit red blood cells. The alcoholic extract was the most potent from the point of view of hemolysis. Acetone and ether extracts were about as potent as the physiological salt-solution extract. In a second series of experiments in which *A. lumbricoides* powder was extracted in the substances referred to above, the extracts were tested on sheep red blood cells. In those experiments the alcoholic extract was the most potent, while the physiological salt-solution extract and the ether extract were the least potent.

Further experiments with different fractions of *Ascaris lumbricoides* were performed. Dried worm material was ground up in a mortar and extracted in four volumes of ether in a flask for 48 hours at 37° C. The ether was then removed from the worm material and saved and fresh ether was added to the flask. This was allowed to extract for 24 hours, the ether being removed at the end of that period and added to the first ether extract. To the worm material fresh ether was again added, and after 24 hours of extraction the mixture was filtered. The last ether filtrate was practically free from any extract. A portion of the ether extract was then evaporated and a brownish yellow fatty substance left behind. This substance had the characteristic odor of *A. lumbricoides*. A small quantity of this substance was emulsified in physiological salt solution and tested on washed rabbit blood corpuscles, which it hemolyzed. A second portion of ether extract in solution was shaken with an equal quantity of distilled water and allowed to remain at room temperature for two hours. Two layers—namely, an ether layer (fraction 1) and a water layer (fraction 2)—were separated. The ether layer (fraction 1) was evaporated, and a fatty substance was left behind which was hemolytic to washed sheep corpuscles. A portion of this substance was redissolved in ether, and to this solution an equal quantity of a solution of sodium bicarbonate was added and the mixture was thoroughly shaken. The ether layer (fraction 1a) was removed
and evaporated. A fatty substance free from the characteristic odor of *A. lumbricoides* was left after evaporating the ether. This substance had no hemolytic power. Inasmuch as sodium bicarbonate saponified the free fatty acids in the ether, it is evident that the hemolytic effect of the ether extract free from the water-soluble fraction is due to fatty acids. Flury (1912), in fact, came to the conclusion that the hemolytic power of ascarids is to be ascribed to free fatty acids of which the unsaturated fatty acids are of prime importance. Flury stated furthermore that oleic acid is probably the most active principle of Ascaris hemolysin because of the known hemolytic powers of this substance. The watery layer (fraction 2) was opalescent and contained a thick suspension of a grayish substance which was found to be slightly hemolytic to sheep cells.

The ether extract contains therefore two fractions, (1) a water-insoluble fraction which consists of neutral fats and fatty acids, and (2) a water-soluble fraction, both of which are hemolytic. The composition of the water-soluble substance was not definitely determined. This substance was tested and found to be soluble in 95 per cent alcohol and in hot and cold water. By acidifying a watery solution of the substance and shaking it with an equal volume of ether it was made to go into solution and was recovered in the ether layer. Another portion of the water-soluble substance was salted out from water by adding a few drops of a strong solution of sodium chloride. It rose to the surface, where it formed a thick layer which was insoluble in salt solution. Bondouy (1908, 1910), who experimented with a similar chemical fraction of a species of Strongylus, identified it as consisting of soluble soaps, substances that are known to have hemolytic power.

To recapitulate, an ether extract of *Ascaris lumbricoides* was divided into the following fractions: (1) An ether-soluble and water-insoluble fraction, and (2) a water-soluble fraction. Both fractions were hemolytic, the latter, however, only to a moderate degree. The fatty acid in the first fraction (fraction 1) was saponified. The fatty acid-free fraction which was extracted in ether was not hemolytic. This fraction consists largely of neutral fats. The hemolytic potency of the ether extract of *A. lumbricoides* is therefore due largely to free fatty acid. That the water-soluble part of the ether fraction (fraction 2) is a mixture of soaps is probable.

A portion of the remaining *Ascaris lumbricoides* powder (free from ether-soluble fraction) was extracted in distilled water for 48 hours in an incubator. The mixture was then filtered. The filtrate had a brownish color and a sweetish odor. Tests for proteins were positive. The residue was evaporated at 40° C. A portion of the residue was taken up in salt solution, to which it gave a yellowish coloration. Tested for its hemolytic power on sheep blood corpuscles, it produced rapid hemolysis. The remaining portion of the residue was extracted in 95 per cent alcohol for
24 hours. It was only partly soluble. After filtering off the alcohol, fresh alcohol was added and the extraction continued for 24 hours longer. The alcoholic extracts were evaporated and the residue was taken up with a small quantity of physiological salt solution. Tested for its hemolytic power, the results were strongly positive on sheep erythrocytes. The alcohol-insoluble fraction was not hemolytic even when large quantities were employed.

These experiments are rather significant in view of the fact that they show quite conclusively that the hemolytic potency of *Ascaris lumbricoides* extracts are due not to fatty acids alone but that another substance or substances, soluble in alcohol and water, must be involved.

The experiments described above were repeated several months later with similar results.

Extracts of powdered ascarids in 95 per cent alcohol were made by adding about 6 volumes of alcohol to 1 volume of powder and removing the alcohol by filtration at intervals of two to three days and adding fresh alcohol. After evaporating the filtrates, which were all mixed together, a brownish residue was left behind which was only partly soluble in ether. The ether-soluble portion as well as the ether-insoluble portion was hemolytic. A portion of the powder, free from the alcohol-soluble fraction, was extracted in ether, but when the latter was removed and evaporated no residue was left behind. The remaining portion of the powder free from the alcohol-soluble portion was extracted in physiological salt solution, and this extract when tested on red blood cells was found to be nonhemolytic. These experiments show, therefore, that the hemolytic substances of *Ascaris lumbricoides* are all soluble in alcohol, and confirm the results of the earlier series of experiments with reference to the fact that the ether-soluble fraction of *A. lumbricoides* contains but a portion of the hemolytic substance.

Part of the alcoholic extract was divided into two fractions—namely, an absolute alcohol-soluble fraction and an absolute alcohol-insoluble fraction. The latter was hemolytic, whereas the former showed no hemolytic power.

An ether extract of *Ascaris lumbricoides* powder was redissolved in ether and divided into two fractions by adding acetone in excess, which resulted in the formation of a whitish precipitate. The precipitate was separated from the solution and found to be nonhemolytic. The acetone-ether solution was evaporated and taken up in salt solution. It was also found to be nonhemolytic, whereas prior to precipitation with acetone the ether extract was hemolytic. The precipitate was obtained in quantities insufficient to determine its nature. That it was probably largely lecithin can hardly be doubted. As is known, lecithin in quantities in which it alone produces no hemolytic effect can activate other substances and cause them to produce hemolysis. That this actually occurs in the

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1 The presence of lecithin in ascarids was demonstrated by Flury (1912).
case of the ether-soluble hemolytic substance of *A. lumbricoides* appears probable from the experiments described above.

It should also be stated that a 95 per cent alcohol extract of *Ascaris lumbricoides* developed a precipitate when kept in solution in 95 per cent alcohol at 8° C. This precipitate went into solution when the alcohol containing the extract was transferred to room temperature. The removal of this precipitate by filtering in a refrigerator yielded a whitish substance which had no hemolytic power, nor did the removal of this substance interfere with the hemolytic potency of the extract.

8. **PROPERTIES OF ASCARIS LUMBRICOIDES HEMOLYSIN**

At low temperatures ranging from 6° to 10° C. hemolytic extracts of *Ascaris lumbricoides* lose their potency. Mixtures of extracts and susceptible corpuscles that showed complete hemolysis after 2 hours' incubation at 37° showed no trace of hemolysis after 24 hours at 8°. After being removed from the low temperatures and transferred to an incubator hemolysis occurred rapidly in such mixtures.

In order to determine whether the hemolytic substance of *Ascaris lumbricoides* is absorbed by the red blood cells at low temperatures the following experiments were performed.

Mixtures of washed red blood cells (rabbit and sheep) and hemolytic extracts were put in a refrigerator at 8° C. After 24 hours the supernatant fluid was removed from the corpuscles and the latter were washed three times in succession to free them from traces of extracts; to the washed corpuscles from which the supernatant fluid had been removed an equal quantity of salt solution was added, and the tubes were thoroughly shaken and placed in the incubator. Hemolysis set in slowly. The supernatant fluid which was removed from the corpuscles was also tested as to its hemolytic potency, with inconstant results. In some cases it was found that it had lost its hemolytic potency completely, but in a number of cases it still retained its blood-destroying power. That the potency of the fluid that had been in contact with susceptible corpuscles had been considerably reduced was evident, since it had but slight hemolytic power as compared with that of intact extract. Whether the hemolytic substance in contact with susceptible corpuscles at a low temperature becomes fixed to the cells or whether it is precipitated at a low temperature and escapes removal despite repeated washing has not been determined.

Hemolytic extracts of *Ascaris lumbricoides* are highly resistant to heat. Heating at temperatures ranging from 56° to 60° C. for 30 minutes did not weaken the potency of the extracts. An exposure to 70° for two hours failed to destroy the hemolytic substance. Salt-solution extract as well as alcoholic extracts were heated to boiling, and after cooling they were tested on susceptible red blood cells. It was found that as a
result of boiling the potency of the extracts was weakened but not destroyed.

The hemolysin goes through the pores of Berkefeld, Chamberland, and diatomaceous filters. The filtrates are less potent, however, than nonfiltered solutions.

V. EXPERIMENTS WITH AGGLUTINATING SUBSTANCES FROM ASCARIS LUMBRICOIDES

In the course of experiments on hemolysis of red blood cells by extracts of *Ascaris lumbricoides* it was observed that the cells frequently became agglutinated before hemolysis set in. The agglutinating effect of the extracts was especially marked on rabbit red blood cells and was observed only occasionally on sheep erythrocytes. Several experiments on hog erythrocytes showed them to be refractory to the agglutinating substance of the parasite.

The agglutinating property of *Ascaris lumbricoides* with respect to rabbit-blood corpuscles was present almost invariably in physiological salt-solution extracts. Alcohol and ether extracts of entire worms were not entirely free from agglutinating properties, however. Unlike the hemolytic substances which are entirely removed from the worm material by alcohol and ether extraction, the agglutinating substance resists extraction in these solvents and may be recovered in the fraction of the worm material from which the alcohol-soluble and ether-soluble fractions have been removed. The salt-solution-soluble hemagglutinin does not appear as firmly bound to the cells of the parasites as the lipoidal hemolysin. The latter, as has already been stated elsewhere in this paper, is but slightly soluble in physiological salt solution unless the material is thoroughly triturated. Salt-solution extracts of coarsely powdered worm material that yield but a small quantity of hemolysin were found to contain a considerable quantity of agglutinating substance. In physiological salt-solution extracts of *Ascaris lumbricoides* that contain the hemolysin and the hemagglutinin the potency of the former may be suppressed by low temperatures (6° to 10° C.), whereas that of the latter remains unaffected by those temperatures.

The hemagglutinin from *Ascaris lumbricoides* is relatively thermostabile and differs in this respect from the hemagglutinin which Tallqvist (1907) isolated from *Diphyllobothrium latum*. The latter is injured by 30 minutes' heating at 55° C., whereas that of *A. lumbricoides* withstands heating at temperatures ranging from 56° to 60° for 30 minutes. Hemagglutinating extract of *A. lumbricoides* was passed through a Chamberland filter without injuring its potency.

Summarizing, it may be stated that in contrast to the lipoidal hemolysin, which is inactive at 6° to 10° C. and which is but slightly soluble in physiological salt solution, the agglutinin of *Ascaris lumbricoides* is readily soluble in salt solution, slightly soluble in ether and alcohol, and
active at low temperatures. It also differs from the hemolysin in its relative specificity for certain species of erythrocytes.

VI. THE EFFECT OF ASCARIS LUMBRICOIDES FLUID ON COAGULATION OF BLOOD

As has already been stated, Weil and Boyé (1910) found that as a result of injecting the fluid of *Ascaris equorum* into rabbits the coagulation of the blood was retarded by 20 minutes. These investigators state, however, that they obtained negative results with rabbit blood in vitro. Leroy (1910) likewise observed that the blood of dogs which had received injections of the body fluid of *A. equorum* exhibited a delayed coagulation time. Flury (1912) made observations on the coagulation of dog blood in contact with the fluid of ascarids in vitro and records a decided delay. His experiments with human blood were likewise positive.

In view of the contention of Weil and Boyé with reference to rabbit blood in contact with *Ascaris equorum* fluid in vitro, the writer tested freshly drawn rabbit blood to which various quantities of *A. lumbricoides* fluid were added, in order to determine if the coagulation power would be affected. The addition of 3 to 5 drops of the fluid to 10 drops of blood delayed the coagulation time about 15 minutes as compared with that of normal blood. The addition of 8 drops of fluid to 10 drops of blood produced a 35-minute delay, whereas the addition of 10 drops of fluid to an equal quantity of blood resulted in a delay of 42 minutes.

The body fluid of *Ascaris lumbricoides* retards the coagulation of blood in vitro as well as in vivo, but its power in this respect is rather limited.

VII. EXPERIMENTS WITH HOOKWORM HEMOLYSIN (ANCYLOSTOMA CANINUM)

The anemia which occurs in cases of infestation with hookworms has been ascribed to several different factors. The direct abstraction of blood by the parasites, the possible absorption of toxic substances from the digestive tract as a result of the ulceration of the mucosa, hemorrhages following the laceration of the mucosa by the worms (Loeb and his collaborators), and the absorption by the host of hemolysins secreted by the parasites have been advanced as explanations. The last view was accepted as a plausible explanation before any experimental evidence in favor of it had been advanced. That the data with reference to the production of hemolysins by hookworms appear to show that such absorption probably occurs has already been pointed out elsewhere in this paper.
In the following experiments the hemolysin was obtained from about 100 specimens of *Ancylostoma caninum* collected from three dogs. The parasites were put into a bottle containing a physiological salt solution and kept in an ice box for about 24 hours after removal from the hosts, without any apparent loss of vitality.

The extract designated as extract of fresh worms was prepared as follows: The parasites were ground up in a mortar containing a small quantity of a physiological salt solution, and the macerated material was then suspended in about 20 cc. of salt solution, shaken vigorously for a few minutes, and placed in a refrigerator overnight. The supernatant fluid was found to be hemolytic, as the following experiments will show.

**Experiment 1.**—To each of three tubes containing 0.5 cc. of a 2 per cent suspension of washed dog erythrocytes there were added, respectively, 5, 8, and 10 drops of the extract of fresh worms. As a control, to a fourth tube containing the same quantity of suspension of corpuscles there were added 10 drops of a salt solution. The tubes were shaken thoroughly and placed in the incubator at a temperature of 37° C. At the end of 30 minutes the tube containing 10 drops of the extract showed complete hemolysis. The tube containing 8 drops of extract showed complete hemolysis 15 minutes later, while the tube containing 5 drops of extract showed partial hemolysis at the end of an hour. The control tube showed no hemolysis. The tubes were kept in a refrigerator overnight and no further change was noted.

**Experiment 2.**—To three tubes each containing 10 drops of a 5 per cent suspension of washed sheep corpuscles there were added, respectively, 5, 8, and 10 drops of the extract of fresh worms. It was necessary to incubate the tubes at 37° C. for two hours before hemolysis was produced in any tube. The tube containing 10 drops of extract showed complete hemolysis; the tube containing 8 drops of extract showed partial hemolysis, while the tube containing 5 drops of extract showed no hemolysis. A fourth tube containing 10 drops of corpuscle suspension and 10 drops of salt solution showed no hemolysis. These tubes were kept in a refrigerator overnight with practically no change in results except that hemolysis was complete in the tube containing 8 drops of extract and was faintly indicated in the tube containing 5 drops of extract.

**Experiment 3.**—The extract of fresh worms was tested against washed rabbit corpuscles as in experiments 1 and 2. Ten drops of a 3 per cent suspension of washed corpuscles were completely hemolyzed by 5 drops of extract in 20 minutes at a temperature of 37° C. This experiment was controlled as usual.
Experiment 4.—Twelve drops of extract of fresh worms were heated for 30 minutes at a temperature ranging from 56° to 58° C. The addition of 0.5 cc. of washed dog corpuscles from the same lot as that used in experiment 1 resulted in partial hemolysis after one hour of incubation at 37°. The tube was kept in a refrigerator overnight and showed almost complete hemolysis the next day.

Experiment 5.—A quantity of extract of fresh worms was heated at 60° to 65° C. for 50 minutes. To two tubes each containing 10 drops of extract that had been thus heated there were added 5 drops of rabbit and sheep corpuscles, respectively, of the same concentration as noted in experiments 2 and 3. No hemolysis was produced after two hours' incubation at 37°. The tubes were kept in a refrigerator overnight and showed slight hemolysis the following day.

Experiment 6.—Twelve drops of extract of fresh worms were heated to boiling, and after cooling they were added to 0.5 cc. of suspension of dog corpuscles of the same concentration as in experiment 1 and were incubated for one hour, but no hemolysis was produced. After remaining in an ice box overnight the tube showed but a trace of hemolysis. Similar results were obtained when rabbit and sheep corpuscles were used. Control tubes showed no hemolysis.

A second series of experiments with extracts of fresh worms was performed several weeks later. The details of these experiments follow.

The extract referred to as extract II of fresh worms was prepared by macerating 29 live specimens of Ancylostoma caninum obtained from five dogs shortly after the animals had been killed. The macerated material was suspended in 3 cc. of physiological salt solution, shaken vigorously, and allowed to extract at room temperature for about an hour before it was tested for its hemolytic power. Part of the extract was kept overnight in a refrigerator and was used the following day. The suspension of corpuscles and extract was incubated at 37° C. for periods shown in the table, the results were noted, and the tubes were then placed in a refrigerator for an additional period of 18 hours, when the final results were read.

The data presented in Table III show that rabbit and dog corpuscles are more susceptible to hookworm hemolysin than the corpuscles of swine and cattle. Despite the fact that the latter were not hemolyzed by the extract used in these tests, they are not absolutely resistant to extracts of dog hookworms, as will be shown in another section of this paper.

1 These specimens were washed several times in physiological salt solution.
Table III gives a record of the experiments performed with this extract.

**Table III.—Effect of extract II of fresh worms (Ancylostoma caninum) on washed red blood corpuscles**

<table>
<thead>
<tr>
<th>Kind of corpuscles.</th>
<th>Quantity of extract</th>
<th>Period of incubation</th>
<th>Results at end of incubation period</th>
<th>Results after 18 hours additional in refrigerator (8° C.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>5 drops</td>
<td>1 hour</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Do</td>
<td>8 drops</td>
<td>do</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Do</td>
<td>Control c</td>
<td>do</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>5 drops</td>
<td>2½ hours</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Do</td>
<td>8 drops</td>
<td>do</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Do</td>
<td>Control c</td>
<td>do</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Hog</td>
<td>5 drops</td>
<td>do</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Do</td>
<td>8 drops</td>
<td>do</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Do</td>
<td>Control c</td>
<td>do</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>5 drops</td>
<td>do</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Do</td>
<td>8 drops</td>
<td>do</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Do</td>
<td>Control c</td>
<td>do</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

a +++ indicates marked though incomplete hemolysis. +++ indicates complete hemolysis. — indicates absence of hemolysis.

b 0.2 cc. of a 5 per cent suspension of washed blood corpuscles were used in all experiments summarized in this table.

c Eight drops of physiological salt solution were added to the washed blood corpuscles in order to control the experiment.

The sediment in the tube containing the extract of hookworms when shaken with 3 cc. of physiological salt solution yielded additional hemolysis, as the following experiments will show.

**Experiment 7.**—After the supernatant fluid from the extract (extract II of fresh worms) had been removed the sediment was shaken up with about 3 cc. of physiological salt solution, which was tested against a 5 per cent suspension of washed dog corpuscles from the same lot as that referred to in Table I. Three drops of corpuscles were completely hemolyzed by three drops of the extract after one hour's incubation at 37° C. This experiment was controlled as usual.

**Experiment 8.**—Five drops of the same extract were boiled for about one minute. After cooling, three drops of dog erythrocytes from the same lot as that used in experiment 7 were added and the mixture incubated for 1½ hours at 37° C. No hemolysis was produced. The tube was kept 18 hours in the refrigerator without any change.

**Experiments with extracts of dried worms**

The experiments recorded below were performed with the following extract:

Fifty-eight mgm. of coarsely powdered worm material (Ancylostoma caninum) dried at 37° C. and kept in a small vial for about two years were extracted in 10 cc. of physiological salt solution for several hours. Unlike
the extract of fresh worms, which is opalescent, the extract of powdered material remained quite clear.

Experiment 9.—To four tubes labeled from 1 to 4, each containing 5 drops of a 5 per cent suspension of washed rabbit corpuscles, there were added, respectively, 3, 5, 8, and 10 drops of the extract. To a fifth tube containing an equal quantity of corpuscles there were added 10 drops of physiological salt solution in order to control the results of the experiment. The tubes were incubated for 1 hour at 37° C., and kept for 18 hours longer in a refrigerator, after which the final results were read. Tube 1 showed no hemolysis, while tubes 2, 3, and 4 showed complete hemolysis. The control tube showed no hemolysis.

Additional experiments with the same extract and the same corpuscles showed that the hemolytic action was very slow, since 10 drops of the extract in contact with 5 drops of the suspension of corpuscles failed to produce hemolysis after 2 hours' incubation at 37° C., but after an additional period of 18 hours in a refrigerator the tube showed complete hemolysis, whereas the control tube showed no trace of hemolysis.

Experiment 10.—The extract of dried worms was tested on a 5 per cent suspension of washed corpuscles of cattle and swine as follows: To four tubes each containing 0.2 cc. of corpuscles there were added, respectively, 1, 2, 3, and 5 drops of the extract, and the tubes were incubated for 1 hour. None of the tubes showed hemolysis. After remaining in a refrigerator overnight the following results were noted.

Cattle corpuscles: The tubes containing 1 and 2 drops of the extract showed partial hemolysis, whereas the tubes containing 3 and 5 drops of extract showed complete hemolysis.

Hog corpuscles: No hemolysis was observed in any tube.

The foregoing experiments were controlled as usual.

In the experiments described above the extract was not filtered but was added to the suspension of corpuscles together with some particles of worm material.

In a second series of experiments performed several weeks later it was found that washed rabbit blood corpuscles were unaffected when placed in contact with an extract of dried hookworms, incubated for 3 hours, and then kept in a refrigerator for an additional period of 18 hours. While no record was made as regards the introduction of particles of worm material into the tubes containing the suspension of corpuscles, it is probable that the clear supernatant fluid alone was added.

A repetition of the experiment on a later date yielded the following results.

Experiment 11.—A small quantity of coarsely powdered worm material was extracted in physiological salt solution, filtered, and the filtrate tested on washed rabbit blood corpuscles. No hemolysis was produced. To the material which had thus been extracted a small quantity of physiological salt solution was added, the contents were thoroughly agitated,
and a few drops containing worm particles were added to 0.5 cc. of a 5 per cent suspension of washed rabbit erythrocytes. After one hour's incubation hemolysis was complete. A tube containing corpuscle suspension alone showed no hemolysis. A repetition of this experiment yielded similar results.

From the foregoing experiments it appears that the hookworm hemolysin is firmly bound to the cells of the parasite. In fresh worms a considerable quantity of free hemolysin is probably present in the tissues and fluids of the body, which is absorbed by the salt solution in the course of extraction. Since the sediment of extracts of fresh worms has been found to yield additional hemolysin after the first extraction, it is evident that salt solution does not absorb all the hemolysin present in the worms. The observation of Preti (1908) that tryptic digestion liberates the hemolysin is further evidence of a close union between the hemolysin and the cells of the worm.

3. EXPERIMENTS WITH EXTRACTS OF ALCOHOLIC SPECIMENS

The experiments described below were performed with extracts obtained from specimens of Ancylostoma caninum which had been preserved in alcohol for about three years. Unless otherwise stated the extracts were prepared as follows: The specimens were washed several times in distilled water, dried at room temperature, and powdered in a mortar; 0.1 gm. of the powder was suspended in 10 cc. of an 0.85 per cent solution of sodium chloride and extracted in a refrigerator for about 24 hours. The supernatant fluid was then tested on the washed erythrocytes of rabbit and sheep as follows.

**Experiment 12.**—Five drops of a 5 per cent suspension of rabbit corpuscles plus 3 drops of extract showed complete hemolysis at a temperature of 27°C in 2 hours. Equal parts of extract and corpuscle suspension showed complete hemolysis in 1½ hours. This experiment was controlled as usual.

**Experiment 13.**—Five drops of a 5 per cent suspension of washed sheep corpuscles were mixed with 10 drops of extract and incubated for 2 hours without producing any hemolysis. A similar experiment was performed a few months later with negative results, despite the fact that after incubating the mixtures of corpuscles and extract for 2 hours they were kept in a refrigerator for 18 hours longer.

**Experiment 14.**—Five drops of a 5 per cent suspension of rabbit corpuscles were not hemolyzed by 5 drops of extract.

**Experiment 15.**—A 5 per cent suspension of washed guinea-pig corpuscles resisted hemolysis after remaining in contact for 3 hours at a temperature of 37°C with an extract of alcoholic specimens made by extracting 200 dried specimens in 6 cc. of physiological salt solution and mixing 3 drops of extract with 2 drops of the suspension of corpuscles. Fifteen drops of the extract in contact with 3 drops of the blood suspen-
tion for 2 hours at 37° C. followed by 48 hours in a refrigerator resulted in partial hemolysis. Several controls in which the suspension of corpuscles alone and equal quantities of the suspension of corpuscles and extract were employed showed complete absence of hemolysis.

These experiments indicate that alcoholic specimens are much less potent in their hemolytic action than fresh specimens. This is doubtless due to the loss of hemolytic substance to the alcohol. In confirmation of this view the writer found that dried hookworms from the dog freed from their ether-soluble and alcohol-soluble fractions were not hemolytic to washed erythrocytes of rabbits. The ether-soluble fraction left rabbit corpuscles intact. The alcoholic extract was unfortunately lost before it was tested for its hemolytic potency.

4. EFFECT OF NORMAL SERUM ON HOOKWORM HEMOLYSIN

EXPERIMENT 16.—To each of four tubes containing 0.5 cc. of blood corpuscles from the same lot as that used in experiment 1 there were added 5 drops of fresh hookworm hemolysin described elsewhere in this paper, and 1, 2, 3, and 5 drops of dog serum, respectively. The tubes were incubated for 1 hour at 37° C. No hemolysis was observed in any of the tubes. After the tubes had remained in an ice box overnight it was found that with the exception of the tube to which but 1 drop of serum was added and which showed a faint trace of hemolysis, inhibition of hemolysis was complete.

EXPERIMENT 17.—Five drops of a 5 per cent suspension of washed rabbit corpuscles from a lot which was susceptible to extract of alcoholic specimens were only partially hemolyzed when 3 drops of normal rabbit serum were added. It was also found that as a result of heating the serum for 30 minutes at a temperature of 56° C. the antihemolytic property was neither destroyed nor impaired.

EXPERIMENT 18.—Washed rabbit corpuscles, which were completely hemolyzed when equal parts of a 5 per cent suspension of cells and equal parts of fresh salt-solution extract were mixed and incubated for 20 minutes at 37° C., were found to resist a double quantity of the hemolysin in the presence of various inactivated sera, as follows: In each of three tubes there were placed 5 drops of the suspension of corpuscles, 10 drops of the extract, and 2 drops of heated rabbit, horse, or dog serum (60° to 65° for 30 minutes). The mixtures were incubated for 2 hours without any resultant injury to the blood corpuscles. After having been kept in a refrigerator for 18 hours after incubation, the tubes containing dog and rabbit serum showed faint traces of hemolysis, while the tube containing horse serum showed no hemolysis.

EXPERIMENT 19.—To each of two tubes containing 3 drops of unwashed rabbit blood there were added 7 drops of physiological salt solution. These mixtures were incubated for 2 hours with 5 and 10 drops of fresh extract, respectively, at 37° C. No hemolysis was produced.
tubes were kept 18 hours longer in a refrigerator, with a resultant faint indication of hemolysis. Washed erythrocytes from the same rabbit were highly susceptible to the extract, since 10 drops of a 3 per cent suspension of corpuscles were completely hemolyzed by 5 drops of extract in about 20 minutes.

**Experiment 20.**—To a series of tubes each containing 3 drops of a 5 per cent suspension of washed dog erythrocytes used in an earlier experiment and included in Table I there were added 5 drops of extract II of fresh worms and various blood sera diluted with an equal quantity of physiological salt solution and heated at 59°C for 30 minutes. The data and results of these experiments, including the controls, are given in Table IV.

**Table IV.**—Effects of various sera on hookworm hemolysin

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Kind and quantity of diluted sera.</th>
<th>Results after 3 hours' incubation at 37°C</th>
<th>Results after 18 hours longer in refrigerator</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 drops (horse serum)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3 drops (dog serum)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3 drops (rabbit serum)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>No serum</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

a +++ indicates complete hemolysis. + indicates slight hemolysis. — indicates absence of hemolysis.  
b Three drops of a 2 per cent suspension of washed dog corpuscles and 5 drops of extract II of fresh worms were used in this series of experiments.

5. **Effect of Cold on Hookworm Hemolysin**

**Experiment 21.**—Dog corpuscles which were found to be highly susceptible to an extract of fresh worms at 37°C remained intact after being kept for 5 hours on ice in contact with a quantity of extract sufficient to destroy the corpuscles at 37°C in 30 minutes. The removal of the supernatant fluid following rapid centrifugation showed that it had completely lost its hemolytic potency, since it failed to hemolyze susceptible dog corpuscles after remaining in contact with them for 2 hours at a temperature of 37°C followed by 18 hours at a temperature of about 10°C.

**Experiment 22.**—The foregoing experiment was repeated, substituting susceptible rabbit corpuscles for dog corpuscles, with similar results.

The loss of the hemolytic property of the extract in contact with susceptible corpuscles at a low temperature can not be attributed to a possible injurious effect of cold, since it was found that the hemolytic potency of the extract was not injured after standing directly on the ice for 18 hours. Washed sheep corpuscles were readily hemolyzed by the refrigerated extract, whereas a control tube containing corpuscles alone showed no hemolysis.

**Experiment 23.**—Six drops of dog blood corpuscles from the same lot as that described in experiment 7 were mixed with 10 drops of extract II
of fresh worms and placed on ice for 3½ hours. The mixture was
centrifuged and the supernatant fluid was removed and to it there were
added 2 drops of washed dog corpuscles from the same lot as used in the
first part of the experiment. After 1 hour's incubation followed by 18
hours in a refrigerator the corpuscles remained intact. The corpuscles
from which the supernatant fluid was originally removed were washed
three times in salt solution and then incubated with a small quantity of
salt solution for 1 hour. Complete hemolysis was produced. A control
tube containing a similar quantity of corpuscles without any hemolytic
extract showed no hemolysis when placed in an incubator. While this
experiment appears to indicate that the hemolysin was fixed to corpuscles
and was not removed by repeated washing, this conclusion must be
accepted with caution, because the possibility remains that some frag-
ments of worms which were introduced into the tube together with the
hemolysin may have been responsible for the hemolysis of the corpuscles
after the removal of the supernatant fluid. The fact that the latter had
lost its hemolytic power affords, however, strong presumptive evidence
of an absorption of the hemolysin by the blood corpuscles.

6. DISCUSSION

The results of experiments with reference to the presence of a soluble
hemolysin in hookworms (Necator and Ancylostoma) show quite con-
clusively that when living specimens are macerated in physiological
salt solution they yield a considerable quantity of hemolysin. The latter
is characterized by relative thermolability, nonspecificity, and suscepti-
bility to normal serum, in the presence of which it loses its potency.
So far as its physiological properties are concerned, hookworm hemolysin
resembles streptocolysin, staphylolysin, tetanolysin, and other hemoly-
sins of bacterial origin. It differs from the hemolytic substances of
Diphyllobothrium latum in that it is destroyed by boiling. The con-
clusion of Preti (1908) that hookworm hemolysin is resistant to boiling
is not sustained by Whipple (1909) and is also contradicted by the
results of the present writer's experiments. Unfortunately, Preti has
not published a full account of his experiments. His general conclusions
are unsupported by details, and judging from the statements that he
makes it does not appear that he controlled his experiments.

The present writer's experiments indicate that the hookworm hemolysin
is rather firmly bound to the tissues of the parasites, which probably
accounts for the difficulty of obtaining strong hemolytic filtrates from
salt solution extracts of powdered specimens. That the living worm
secretes the hemolysin is evident, however, from experiments with ex-
tracts of fresh worms. The unbound hemolysin from fresh specimens
evidently disappears in the course of drying. This comparative insolub-
ility of the hemolytic substance from dried specimens in physiological
salt solution is perhaps the basis of the contention of Preti (1908) and of
Usami and Mano (1918) concerning the insolubility in water of the hookworm hemolysin. That Loeb and his collaborators used dried material has already been stated.

The fact that normal blood serum has antilytic properties and inhibits the action of the hookworm hemolysin accounts for the negative results obtained by Loeb and Smith (1904) and for the weakly positive results obtained by Whipple (1909). In this connection it is important to recall the observations of Noc (1908) with reference to the presence of antihemolysins in the blood serum of normal persons and of those recovering from hookworm disease and from beriberi, and the absence of anti-hemolysins in patients suffering from these diseases. Noc's observations are decidedly significant and do not bear out Whipple's view that the hookworm hemolysin probably bears no relation to the secondary anemia of ancylostomiasis. De Blasi's observations with reference to the presence of hemolysins in the blood serum of patients infected with hookworms and Noc's discovery that under certain conditions the antilytic action of the blood serum may become impaired appear to indicate that the hookworm hemolysin has potentialities of causing anemia and that in severe infections it probably plays an important rôle in the disease.

Since cold (6° to 8° C.) inhibits the action of the hookworm hemolysin in vitro, and the supernatant fluid from tubes in which susceptible blood corpuscles and potent hookworm extract have been in contact for a number of hours at a low temperature no longer has hemolytic properties, the view that the hemolysin is a complex organic substance, not unlike a toxin, in that it apparently consists of haptophore and toxophore groups, appears to be justified. By means of the haptophore group union between the hemolysins and blood corpuscles takes place, but the dissolving or lytic action is produced by the toxophore group. Inasmuch as low temperatures do not appear to interfere with the absorption of the hemolysin by the corpuscles despite the fact that the latter remain undissolved, it is permissible to believe that the toxophore and haptophore groups of the hookworm hemolysin act independently of each other. This view is purely speculative, however, and further experimentation is required before it may be accepted without reservation.

VIII. EXPERIMENTS WITH EXTRACTS OF CATTLE HOOKWORMS (BUSTOMUM PHLEBOTOMUM)

Hookworms belonging to the genus Bustomum occur as parasites in the small intestine of ruminants. Bustomum phlebotomum is the species that infests cattle. According to observations of several investigators, cattle infested with hookworms show symptoms not unlike those of human beings that harbor species of Ancylostoma or Necator.

Experiments with extracts of Bustomum phlebotomum similar to those performed with extracts of Ancylostoma caninum showed that the former, like the latter, contain a powerful hemolytic agent. The extracts
referred to below were prepared as follows: Living worms were removed from the intestine of a calf, washed a number of times in physiological salt solution, and kept in a refrigerator at a temperature of 8° C. overnight. The following day the worms which were still alive were transferred to fresh salt solution and crushed in a mortar. The crushed material was then suspended in about two volumes of physiological salt solution, shaken thoroughly, and centrifuged. The supernatant fluid which was opalescent was removed and tested as to its hemolytic power. Tested on a 3 per cent suspension of washed sheep blood corpuscles, it was found that 5 drops of the extract hemolyzed 5 drops of the suspension of blood corpuscles in 1 hour at a temperature of 37°. Even 1 drop of extract hemolyzed 5 drops of the suspension of corpuscles after a few hours. Controls, that is, 5 drops of suspension of corpuscles plus 5 drops of salt solution, remained intact. It was observed that before hemolysis set in the contents of the tubes assumed a dark red hue.

An extract from another lot of Bustomum phlebotomum prepared as has already been described was tested on a 5 per cent suspension of washed rabbit cells. Five drops of extract produced hemolysis rather slowly upon 5 drops of suspension of corpuscles.

In a third experiment an extract prepared from worms that had been kept in a refrigerator overnight was tested on four different tubes of cattle erythrocytes and on four different tubes of hog erythrocytes. The extract in question was prepared from living specimens as follows: Forty-five specimens were ground up in a mortar and suspended in 2 cc. of physiological salt solution. The suspension was centrifuged, and the opalescent fluid was removed and tested on a 5 per cent suspension of washed blood corpuscles at 37° C. Three drops of extract were added to 5 drops of corpuscle suspension. The experiments with each sample of corpuscle suspension were controlled by adding 3 drops of physiological salt solution to 5 drops of suspension of blood cells. The results of these experiments follow.

**CATTLE BLOOD CORPUSCLES.**—After 1 hour one tube of blood was partially hemolyzed and three tubes were intact. After 2½ hours two tubes were completely hemolyzed and two were intact. After 3 hours three tubes were hemolyzed; one was intact. The tubes containing the mixtures were placed in a refrigerator at 8° C. until the next day. When examined hemolysis was complete in all tubes. The controls showed no hemolysis.

**HOG BLOOD CORPUSCLES.**—After 1 hour all tubes were intact. After 1½ hours two tubes were partially hemolyzed and two intact. After 2½ hours two tubes were partially hemolyzed and two completely hemolyzed. After 3 hours all tubes showed complete hemolysis. Controls were intact.

Inasmuch as it was found that the hemolysin could be preserved by drying the worms, powdering the dried material, and storing it in a dark place, further experiments with Bustomum phlebotomum hemolysin were performed with dried material. The details of these experiments follow.
To each of four tubes of defibrinated blood (3 drops of physiological salt solution plus 2 drops of blood) a small quantity of the powder was added, and the tubes were shaken thoroughly and placed in an incubator at 37° C. After 2 hours hemolysis was produced in all tubes. Two lots of cattle blood from different animals were collected in a 2 per cent solution of sodium citrate (about 2 volumes of blood to 1 volume of a 2 per cent sodium citrate). Tested against dry _Bustomum phlebotomum_ powder the unwashed citrated blood became hemolyzed in about 2 hours at 37°.

Small quantities of powder were also tested on each of four lots of washed cattle blood corpuscles with positive results. Hemolysis set in rapidly and was complete after 1 hour at 37° C.

A few drops of a 3 per cent suspension of washed sheep corpuscles were hemolyzed by a small quantity of _Bustomum phlebotomum_ powder. Similar results were obtained with washed rabbit erythrocytes.

_Bustomum phlebotomum_ powder extracted in physiological salt solution yields but a small quantity of hemolysin, as the following experiments will show.

Eighty-five mgm. of powder were suspended in 5 cc. of physiological salt solution. A few drops of chloroform were added as a preservative. The mixture was kept at a temperature of 35° to 37° C. for 2 days and then filtered. The clear filtrate was tested on a 5 per cent suspension of washed rabbit cells. Equal parts of filtrate and suspension of cells yielded negative results. It was necessary to add 10 drops of filtrate to 3 drops of corpuscle suspension to produce hemolysis. Evidently the hemolysin is firmly bound to the parasite material and is but slightly soluble in salt solution. In fact, the powder which had been extracted was dried and retested on rabbit blood cells, which it hemolyzed rapidly.

An alcoholic extract of fresh specimens of _Bustomum phlebotomum_ was found to be decidedly hemolytic. The extract was prepared as follows: About 100 specimens were washed a number of times in physiological salt solution after they had been removed from the host. The specimens were then triturated in a mortar and extracted in about 2 volumes of 95 per cent alcohol for about a week at 37° C. The alcohol was separated from the worm material by filtration. The filtrate was evaporated and the residue was shaken with a small quantity of physiological salt solution, in which it dissolved, producing an opalescent solution. Tested on sheep red blood corpuscles this solution produced hemolysis. A quantity of the solution which hemolyzed 5 drops of a 5 per cent suspension of washed sheep corpuscles in about 2 hours at 37° failed to produce hemolysis on an equal quantity of blood corpuscles in 20 hours in a refrigerator (8°), thus showing that low temperatures paralyze the action of the hemolysin. Likewise, normal horse serum (2 drops) inhibited hemolysis of 5 drops of washed sheep corpuscles to which sufficient hemolytic solution had been added to cause hemolysis in the absence of
normal serum. The worm material from which the alcohol-soluble sub-
stance had been removed was dried and pulverized. A portion of this
powder was added to washed sheep corpuscles but failed to produce any
hemolytic effect, showing that the hemolytic substances of Bustomum
phlebotomum are completely soluble in alcohol.

In a few experiments the effect of normal serum was tested with a
view of determining when it contained bodies capable of inhibiting the
action of Bustomum phlebotomum hemolysin. Washed rabbit corpuscles,
belonging to a lot that were rapidly hemolyzed by a small quantity of
the powder, resisted hemolysis in the presence of a few drops of normal
rabbit serum.

The effect of heat on the hemolysin was found to be the same as the
effect of heat on Ancylostoma caninum hemolysin. A salt-solution
extract of fresh worms was completely inactivated by heating it for 40
minutes at 60° C.

IX. EXPERIMENTS ON THE POSSIBLE PRESENCE OF ANTICOAGULINS
IN HOOKWORMS

A series of experiments was performed with a view of determining
whether the two species of hookworms discussed in the foregoing pages
(Ancylostoma caninum and Bustomum phlebotomum) secrete a substance
that has the power of inhibiting the coagulation of rabbit blood. Salt-
solution extracts of fresh and dried material from the two species were
tested as follows.

Into a series of tubes containing varying doses of extract, rabbit
blood drawn directly from the marginal ear vein was allowed to drop. Each experiment was controlled by allowing an equal quantity of blood
to drop into tubes containing physiological salt solution. So far as the
rapidity of coagulation of the blood was concerned, appreciable but not
very marked differences were detected between the test and control
tubes. These experiments were performed on the blood of several
rabbits with uniformly negative results.

Inasmuch as Loeb and his collaborators tested the anticoagulin from
Ancylostoma caninum on dog blood and obtained positive results, it would
appear that the writer's negative results may indicate that the anticoag-
ulins in hookworms are either strictly specific for the blood of their host
or that they are perhaps only relatively specific. Further data bearing
on hookworm anticoagulin as well as anticoagulins from other nematodes
are given in a separate paper (Schwartz, 1921).

X. EXPERIMENTS WITH EXTRACTS OF HAEMONCHUS CONTORUTS

Haemonchosis or stomach-worm disease is a disease of cattle and sheep
due to the presence in the fourth stomach of a nematode parasite known as Haemonchus contortus. Young animals are especially susceptible to
stomach-worm disease, and among other symptoms they show those of a rather severe anemia. As in hookworm disease, the direct abstraction of blood by the parasites undoubtedly plays a part in bringing about the train of morbid symptoms associated with loss of blood, but that other factors are involved—namely, a chronic intoxication of the host by toxic substances liberated by the parasites—appears probable. Furthermore, it is by no means unlikely that as the susceptible animals grow older they become more or less immune to the effects of the parasites, although they are by no means immune to infestation with the worms. Whether the immunity is developed as a result of a previous infestation or whether it is a natural immunity associated with maturity is not known. In fact the clinical phase of haemonchosis is still an almost unexplored field in parasitology.

The following experiments were performed by the present writer with reference to the presence of a soluble hemotoxin in this parasite.

A number of specimens of *Haemonchus contortus* (about 100) that had been removed from a calf shortly after death were washed a number of times in physiological salt solution and kept in a refrigerator overnight. The following day the specimens were still alive. They were ground up in a mortar with a small quantity of physiological salt solution. The crushed material was transferred to a test tube and allowed to remain at room temperature for about two hours. The supernatant fluid was then tested on a 5 per cent suspension of washed sheep corpuscles. After 2 hours at $37^\circ$ C. a number of tubes containing graded quantities of extracts and 5 drops of washed red blood cells showed no trace of hemolysis. The tubes were then transferred to a refrigerator, where they remained 18 hours longer. A faint trace of hemolysis was present in the tube containing the largest quantity of extract. The control tube was intact.

A second experiment of a similar nature was performed with another lot of fresh worms. In this case the extract was tested on washed sheep blood corpuscles, with negative results. Alcoholic specimens of *Haemonchus contortus* from sheep were washed in salt solution to remove traces of the alcohol and then dried at $37^\circ$ C. The dried material was pulverized, and part of it was extracted in salt solution and tested on washed sheep corpuscles, with negative results. The remaining portion of the dried material was extracted in 95 per cent alcohol and the extract suspended in salt solution. Tested on sheep corpuscles, this extract likewise yielded negative results.

A number of fresh specimens of *Haemonchus contortus* were dried at $37^\circ$ C. and pulverized in a mortar. Graded quantities of the powder were added to washed sheep blood corpuscles. After 2 hours at $37^\circ$ followed by 18 hours in a refrigerator slight hemolysis was produced.
To one tube a small quantity of carbolized horse blood serum was added. The serum inhibited hemolysis.

_Haemonchus contortus_ powder was also tested on four samples of washed cattle blood cells. The results were slightly positive after 2 hours at 37° C. followed by 18 hours in a refrigerator.

Inasmuch as in the experiment described above washed red blood cells were used, a series of tests were performed in which unwashed defibrinated blood was used. In this series six samples of cattle blood were involved. The addition of various quantities of _Haemonchus contortus_ powder yielded negative results after 3 hours at 37° C.

Summarizing, salt-solution extracts of _Haemonchus contortus_ are very slightly hemolytic to sheep and cattle erythrocytes. The faint hemolytic property is preserved by drying. The weakly positive results obtained by experiments in vitro do not favor very strongly the view which has been commonly accepted as regards the secretion of a hemotoxin by _H. contortus_. It is quite possible, however, that the apparently weak hemolysin requires some activator which is supplied by the host blood. The fact that experiments in vitro were only slightly positive by no means precludes the possibility that an absorption by the host of the secretions of _H. contortus_ is followed by a marked hemolysis. Another possibility, which has already been mentioned, is that only the blood of young animals is susceptible to the secretions of _H. contortus_. The subject requires further investigation.

**XI. EXPERIMENTS WITH TRICHURIS DEPRESSUSCULA EXTRACT**

A small series of experiments with an extract of _Trichuris depressuscula_ was performed as follows: About 60 specimens collected from several dogs were thoroughly washed in physiological salt solution and dried in an incubator. The dried specimens were then triturated and extracted in 3 cc. of salt solution overnight at 8° C. The clear filtrate was tested on rabbit and sheep erythrocytes. Five drops of a 5 per cent suspension of rabbit blood cells were hemolyzed by 3 drops of extract in about 2 hours at 37°. Equal mixtures of sheep erythrocytes and extract showed no hemolysis. The extract was boiled for about a minute, and after it had cooled it was tested on rabbit erythrocytes. It produced a faint indication of hemolysis, showing that boiling practically destroyed the hemolysin.

**XII. EXPERIMENTS WITH CESTODE HEMOLYSINS**

It has already been stated that while an active hemolytic agent has been shown to occur in _Diphyllobothrium latum_, evidence that other species of tapeworms secrete hemolytic substances is rather incomplete. The presence of a hemolytic agent in _D. latum_ is significant in view of the fact that this parasite is capable of producing a severe anemia under

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1 0.25 per cent solution of carbolic acid in serum.
certain conditions that are not yet understood. Inasmuch as cestodes are not capable of causing anemia by direct abstraction of blood or by lacerating the mucosa, the etiological rôle of a hemotoxin, if such a substance can be demonstrated in forms that cause anemia, can hardly be denied. The discovery of Schaumann and Tallqvist (1898) and the subsequent studies of Tallqvist (1907) and Faust and Tallqvist (1907) with reference to the *D. latum* hemolysin are of great significance and mark the beginning of the study of the pathogenicity of parasitic worms from the point of view of intoxication. Despite the fact that *D. latum* appears to stand alone among cestodes capable of setting up a severe type of anemia, there is some evidence that other cestodes are also capable of bringing about anemia, perhaps not so intense as that produced by *D. latum*. Railliet (1895), Neveu-Lemaire (1912), Hutyra and Marek (1913), and other writers on veterinary parasitology state that cattle and sheep that are parasitized by tapeworms show clinical symptoms of anemia. Adult cestodes parasitic in these ruminants belong to the genera *Moniezia* and *Thysanosoma*. Only one species of the latter genus is known in the United States, namely, *Thysanosoma actinioides*, whereas several species of *Moniezia* occur in this country. Investigations by the present writer with reference to hemolysins in worms belonging to the genera *Moniezia* and *Thysanosoma* have yielded the following results.

A salt-solution extract of *Thysanosoma actinioides* powder made by adding the powder to salt solution and allowing the extract to remain at 8° C. for about 24 hours was found to be hemolytic to washed sheep blood cells and rabbit blood cells. In one experiment 150 mgm. of powder were extracted in 5 cc. of physiological salt solution overnight at a temperature of 8°. The supernatant fluid was filtered and the filtrate tested on washed rabbit blood cells. Equal parts of extract of suspension of corpuscles showed complete hemolysis after 2 hours at 37°. Further experiments with salt-solution extracts of dried material on washed sheep and rabbit blood corpuscles confirmed the presence of a soluble hemolysin in this parasite. Thus, an extract prepared by adding 0.2 gm. of powder to 2 cc. of salt solution was tested on rabbit and sheep blood corpuscles and yielded positive results. The action of the hemolysin was comparatively slow. To tubes each containing 5 drops of washed blood cells 5 and 10 drops, respectively, of the extract were added and incubated at 37° for 2 hours; hemolysis was not evident in the tubes. After an additional period of 18 hours during which the tubes were kept in a refrigerator hemolysis was complete in the tube to which 10 drops of extract had been added and marked but incomplete in the tubes to which only 5 drops of extract had been added. It should be stated in this connection that in several instances salt-solution extracts of dried *T. actinioides* were not destructive to red blood cells of sheep. Whether the red blood cells of certain animals are more resistant than
others, or whether the different extracts used in these experiments varied in their hemolytic content, has not been determined. At any rate, salt-solution extracts of *T. actinioides*, so far as the experiments referred to above are concerned, are not strongly hemolytic.

A quantity of *Thysanosoma actinioides* powder was extracted in four volumes of ether. The ether extract after it had been freed from all traces of ether was emulsified in physiological salt solution and tested on sheep blood corpuscles with positive results. After a second extraction of the powder in ether a quantity of powder free from the ether-soluble fraction was extracted in physiological salt solution, and this extract was found to be nonhemolytic. The remaining powder was extracted in 95 per cent alcohol. After filtration the alcohol was evaporated, and the residue, which had a waxy appearance and consistency, was dissolved in physiological salt solution and tested on sheep red blood cells with positive results. Boiling did not destroy the hemolytic potency of this extract; neither did cold inhibit its activity. Normal horse serum inhibited its action completely.

After alcohol extraction the powder was extracted in physiological salt solution and tested on sheep red blood cells. It was only faintly hemolytic.

Another lot of powdered *Thysanosoma actinioides* was extracted in 95 per cent alcohol three times in succession, each extraction lasting 48 hours. After the last extraction only a slight residue was left when the alcohol had completely evaporated. The residues were dissolved in physiological salt solution and tested on sheep and rabbit blood cells with positive results. Boiling did not destroy them and low temperatures had no inhibiting effect on them. The powder freed from the alcohol-soluble fraction was extracted in salt solution, and this extract was nonhemolytic.

It may be concluded, therefore, that a hemolysin is present in *Thysanosoma actinioides*, soluble to some extent in physiological salt solution and completely soluble in alcohol. Ether extracts of *T. actinioides* are hemolytic, but worm material freed from ether-soluble fractions still retain the hemolytic agent. That substances other than fatty acids are involved in the hemolytic effects of *T. actinioides* extracts is evident, since the ether extracts remove whatever fatty acids are present in the worms. The alcohol-soluble and ether-insoluble fraction of *T. actinioides* resembles rather closely tissue lysins so far as the chemical and physiological properties of tissue lysins are known. Noguchi (1907) found that tissue lysins are soluble in 95 per cent alcohol, are not removed by ether extraction, and that they have the chemical properties of soluble soaps. In common with the latter they are destructive to red blood cells even at 0°C, are neutralized by normal serum, and are resistant to boiling. While the chemical nature of the ether-insoluble and alcohol-soluble fraction of *T. actinioides* has not been determined, its resem-
blance to tissue lysins appears to be very close. The *Ascaris lumbricoides* hemolysin as well as the *Bustomum phlebotomum* and *Ancylostoma caninum* hemolysins are not active at low temperatures, as shown elsewhere in this paper.

Experiments with a species of Moniezia similar to those performed with *Thysanosoma actinioides* have yielded negative results. The addition of various quantities of powdered Moniezia material to suspension of washed red blood cells of rabbit and sheep produced no destructive action on the cells. A salt-solution extract of Moniezia powder was likewise nonhemolytic when tested on washed sheep blood cells. An ether extract was only slightly hemolytic, but after removing from the ether extract the acetone-insoluble fraction, presumably lecithin, its hemolytic potency was no longer manifest. The acetone-insoluble fraction had no destructive effect on sheep blood corpuscles. A quantity of Moniezia powder freed from the ether-soluble fraction by repeated extraction with ether was extracted for 72 hours in 95 per cent alcohol at 38° C. The alcohol was separated from the alcohol-insoluble powder by filtration and evaporated. The residue was taken up in physiological salt solution, in which it was only partly soluble, the insoluble portion forming a coarse suspension. This solution had a decidedly acid reaction. Tested on washed sheep red blood corpuscles, it produced no hemolytic effect.

**XIII. RESULTS OF EXPERIMENTS**

The data presented in the foregoing pages have already been summarized in connection with each separate topic. The discussion which follows is for the purpose of correlating, comparing, and differentiating the results obtained with various species of parasitic worms that have been referred to in this paper, and to consider the general bearings that the results have on the nature of parasitic infection.

Hemotoxins present in parasitic worms contain one or more active principles. Of the latter, hemolysins stand out as of prime importance. Hemagglutinins and anticoagulins may be associated with hemolysins.

Hemagglutinins have thus far been observed in *Diphyllobothrium latum* by Tallqvist (1907) and in *Ascaris lumbricoides* by the present writer. Tallqvist describes the hemagglutinin from *D. latum* as a water-soluble, alcohol- and ether-insoluble substance, decidedly thermostable. The hemagglutinin observed by the present writer in extracts of *Ascaris lumbricoides* is resistant to heat and soluble in lipid solvents, such as ether and alcohol, as well as in physiological salt solution. It is, therefore, quite a different substance from the agglutinin of *D. latum*. Anticoagulins have been found in species of Strongylus (Weinberg, 1908), in the larvae of *Gastrophilus* (Weinberg, 1908), in species of *Ascaris* (Weil and Boyé, Leroy, and the present writer), in *Ancylostoma caninum* (Loeb
and his collaborators), and in several other species by the present writer
(1921.) The anticoagulin in Ancylost. i caninum is the most active of
the anticoagulins observed in parasitic worms and is doubtless a factor
in the anemia that is present in hookworm disease. The anticoagulin of
Ascaris lumbricoides has but a feeble action, so far as available experi-
mental data show.

Hemolysins from parasitic worms, so far as they have been described
in the literature, have certain properties in common with hemolysins of
bacterial origin as well as with hemolysins that have been obtained from
normal tissues by Korschum and Morgenroth, Noguchi, and others.
These properties may be characterized as nonspecificity in action and
relative simplicity of structure as compared with hemolysins that may
be artificially produced in animals by immunization with red blood
corpuscles. The experiments recorded in this paper do not in any case
contradict these facts. Different species of blood corpuscles may show
differences in resistance to hemolytic extracts of parasitic worms, but
absolute resistance of any species of corpuscles has not been established.
Similarly, extracts from different parasitic worms differ in their resist-
ance to heat, but once their potency has been destroyed it can not be
reactivated by normal serum. The only apparent contradiction to this
statement is the result of a small series of experiments of Garin with
Graphidium strigosum, which, as has already been indicated, can not
be accepted as conclusive in view of the small number of experiments.
Hemolysins produced in an animal as a result of immunization with
red blood corpuscles, are, as is well known, specific in their action,
affecting only corpuscles against which the animal has been immunized,
and complex in structure, since they act in combination with comple-
ment and may be reactivated by normal serum after the complement
has been destroyed.

So far as their resistance to heat is concerned, hemolysins from para-
sitic worms differ considerably. Heat-resisting hemolysins have been
recorded by Tallqvist from Diphyllobothrium latum, by Weinberg from
species of Strongylus, and by the present writer from Ascaris lumbricoides
and Thysanosoma actinioides. Hookworm hemolysins from worms of the
genera Ancylostoma, Necator, Bustomum, and the hemolysin from Tri-
churis depressiuscula are not as resistant to heat. The relatively thermo-
labile hemolysins from these parasites resemble in this respect bacterial
hemolysins, whereas the thermostabile hemolysins resemble in this respect
tissue extracts.

The solubility of hemolysins from parasitic worms in lipoid solvents,
especially in alcohol, is another property that they have in common with
tissue lysins. A property of the latter is also the nonimpairment of their
activity at low temperatures, even at 0° C. So far as the results of
experiments recorded in this paper are concerned, hemolysins from
worms belonging to the genera Ascaris, Ancylostoma, and Busostomum are inhibited at 8°. The hemolytic effect of Thysanosoma actinioides extract is not inhibited at this temperature, however. This fact is important and clearly differentiates hemolysins of nematodes from the hemolysin of T. actinioides. In this respect, too, nematode hemolysins resemble bacterial hemolysins.

Finally, the action of hemolysins from parasitic worms is inhibited by normal serum. The antilytic property of the serum is thermostabile (Weinberg, 1908, and the experimental results obtained by the present writer). Tissue lysins, too, are inhibited by normal serum. Certain bacterial hemolysins are similarly susceptible to normal serum.

On the basis of this discussion nematode hemolysins may be characterized as relatively simple substances, thermolabile or thermostabile, depending on the species from which they are obtained, inactive at low temperatures (6° to 8° C.), inactive in the presence of normal serum, nonspecific, soluble in alcohol and in physiological salt solution.

Cestode hemolysins, so far as they have been investigated, are relatively simple bodies, thermostabile, active at low temperatures, inactive in the presence of normal serum, nonspecific, soluble in alcohol.

The question naturally arises whether toxic products from parasitic worms are liberated from the bodies of the latter and get into the circulation of the host. Blanchard (1905), while accepting the evidence in favor of the view that parasitic worms elaborate toxic products, appears to doubt the etiological significance of these toxic substances because of the possibility that they are either not liberated by the worms or if liberated may be thrown out of the body before they can injuriously affect the host. The available evidence on this question appears to indicate that hosts harboring parasitic worms actually absorb the toxic products of the latter. The serological evidence in favor of this view has already been referred to. It may be added that the fact reported by Guerrini (1908) with reference to the presence of hemolysins in the blood serum of hosts harboring Fasciola hepatica and the findings of De Blasi that hemolysins occur in the blood serum of hosts harboring Ancylostoma duodenale tend to confirm the belief that parasites liberate their toxic secretions and that these secretions get into the circulation of the host. Weinberg (1908) has made some interesting observations on the tissues of hosts harboring parasitic worms which argue directly in favor of the absorption by the host of toxic products liberated by the worms. Weinberg examined histologically the organs of 32 horses infested with strongyles and obtained the following results: In the blood vessels he found a large number of mononuclear leucocytes containing iron granules. He also found similar granules in the spleen, liver, in the conjunctival tissue, in the Malpighian tubules and in the convoluted tubules of the kidneys, and in the canals of the right kidney. Histological examinations by the same investigator of organs from 30 monkeys infested with a species of
Cesophagostomum yielded similar results. In another paper Weinberg (1909) records that the injection of extracts of worms of the genus Strongylus into guinea pigs leads to a pigmentation of the spleen but seldom of the liver. From this, it appears that erythrocyte destruction takes place in animals that harbor hemotoxin-producing parasitic worms and that the disintegration products of the erythrocytes are ingested by leucocytes, arrested in certain organs, and eliminated through the excretory system.

Whether the hemotoxic substances from parasitic worms are liberated during the normal life of the worms, or whether they are liberated only when worms sicken or degenerate, as appears to be the case in *Diphyllobothrium latum*, cannot as yet be stated with certainty. In the case of *D. latum* the view that only certain specimens secrete the hemolysin has been advanced by a number of investigators, especially by Leichtenstern (1896). Tallqvist's experiments show that hemolysins are present in specimens of *D. latum* expelled from patients that show no symptoms of anemia as well as in specimens obtained from cases of severe anemia. Tallqvist's hypothesis that the hemolysin is eliminated when the worms disintegrate finds confirmation in numerous cases in which eggs of *D. latum* are present in the feces of patients, and anthelmintic medication fails to expel any worms and merely yields a mass of eggs. Another factor which may be of importance, and which, so far as the present writer is aware, has been entirely overlooked, is the fact that certain individuals may lack antilytic constituents in their blood and are thus susceptible to the toxin which other individuals are capable of neutralizing. That the antilytic properties of the blood may under certain conditions be absent is probable from the observations of Noc (1908) with reference to hookworm disease. Whether the observations with reference to *D. latum* are applicable to other parasitic worms, especially to nematodes, can not in the light of our present knowledge be stated with any degree of certainty. That parasites may die in the intestine or other location and disintegrate before they are eliminated from the body of the host is by no means improbable. Cultures of larvae of parasitic worms in vitro show that bacteria may kill the worms, and that the latter undergo degenerative changes, such as complete internal disorganization, quite rapidly. That worms may be attacked by bacteria and other organisms in the body of the host is by no means improbable. Weinberg has in fact described what appears to be a disease in worms belonging to the genus Ascaris, which is characterized by the presence of certain pigmented spots that are clearly visible through the cuticle. The present writer has observed this condition in specimens of *Ascaris lumbricoides* on several occasions.

Whether parasitic worms liberate their toxic secretions during life or whether these substances partake of the nature of endotoxins and are not liberated from the bodies of the worms unless the latter disintegrate
is still open to speculation, but the view that toxic substances from parasitic worms are of etiological significance in parasitic diseases is supported by convincing evidence.

XIV. SUMMARY

Extracts of *Ascaris lumbricoides* contain active substances that affect blood deleteriously. The hemolysin which these extracts contain is a thermostable, nonspecific, alcohol-soluble substance which appears to be rather firmly bound to the cells of the parasite, presumably to the cells of the intestine in which it is elaborated. The hemolytic potency of extracts of *A. lumbricoides* is not due solely to fatty acids, since chemical fractions of the worms from which the fatty acids have been removed by ether extraction are hemolytic. The hemolysin is neutralized by normal blood serum.

The body fluid of *A. lumbricoides* shortly after removal from the host contains oxyhemoglobin and is nonhemolytic. It acquires hemolytic powers, however, as the worms are kept alive in vitro for a few days, and loses at the same time its oxyhemoglobin content.

Body fluid from fresh specimens of *Ascaris lumbricoides* does not activate a hemolytic system, and alcohol-soluble fractions of the worms from which ether-soluble substances have been removed does not act as complement in combination with inactivated normal guinea-pig serum.

The hemagglutinin from *Ascaris lumbricoides* is a salt-solution-soluble substance and has special affinities for rabbit blood cells. Unlike the hemolysin, its action is not hindered by low temperatures (6° to 8° C.).

*Ascaris lumbricoides* secretes a substance that inhibits the coagulation of blood. This substance is present in the body fluid of the worm and has but a comparatively slight potency.

*Ancylostoma caninum* secretes a nonspecific hemolysin, soluble in salt solution, relatively thermostable and inactive at low temperatures. Normal blood serum inhibits the action of the hookworm hemolysin.

*Bustomum phlebotomum* secretes a hemolysin having properties similar to that of *Ancylostoma caninum*. This hemolysin is completely soluble in alcohol.

Salt-solution extracts of *Haemonchus contortus* have but a feeble hemolytic action.

Salt-solution extracts of *Ancylostoma caninum* and of *Bustomum phlebotomum* do not inhibit the coagulation of rabbit blood to any marked degree.

A weak hemolytic substance is present in extracts of *Trichuris depressi-uscula*.

*Thysanosoma actinioides* contains an alcohol-soluble hemolysin. Alcohol-soluble fractions of *T. actinioides* from which the ether-soluble fraction has been removed are hemolytic, showing that substances other than fatty acids are involved. The hemolysin from this cestode is active at 8° C.
and is neutralized by normal blood serum. Extracts of a species of Moniezia similar to those of Thysanosoma actinioides are nonhemolytic.

The view that hemolysins and other hemotoxic secretions of parasitic worms are of etiological importance in parasitic diseases appears to be well founded.

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