Filaroides hirthi: Experimental Transmission Among Beagle Dogs Through Ingestion of First-Stage Larvae

Abstract. *Four beagle dogs were found to be infected with sexually mature* Filaroides hirthi *lungworms from 1 to 3 months after they ingested material containing first-stage larvae of this parasite. Infection by first-stage larvae opens the theoretical possibility of autoinfection of which few nematode parasites are capable.*

Infections with the lungworm *Filaroides hirthi* are of great practical importance because (i) the parasite is enzootic in all five of the major research beagle breeding establishments in the United States (*1*); (ii) the associated lesions may be confused with pathological changes induced by drugs, oncogenic influences, and other pathogenic organisms (*2*); and (iii) antemortem diagnosis is impossible because larvae cannot be demonstrated in the blood, secretions, or excretions of infected beagle dogs (*2*, *3*). Infection with *F. hirthi* is thus widespread in beagle dogs used for research and causes lesions that interfere with the pathological evaluation of experimental material. Transmission of this infection is certainly efficient because the infection rate is virtually 100 percent in at least one of the affected establishments (*3*). The diet fed at this establishment was suspected to be the source of infection because it contained uncooked meat. However, when fed for 14 weeks to ten uninfected beagle pups as the only form of nourishment, this diet failed to produce a single infection (*3*). In addition, I have identified *F. hirthi* infection in beagle dogs that were raised from weaning exclusively on commercial dried dog food.

Dorrington (*4*) reported success in transmitting *Filaroides osleri* infection by feeding dogs with first-stage larvae obtained from nodules in the trachea and bronchi of infected dogs. Dunsmore and Spratt (*5*) confirmed this by infecting captive dingoes, dingo–domestic dog crosses, Labrador dogs, and a fox with first-stage larvae, "some hatched, others unhatched." This is an exceptional result because all but a very few nematode parasites (*6*) require a period of development outside of the definitive host or as parasites of an alternate host. For example, all members of the superfamily Metastrongyloidea (to which the genus *Filaroides* belongs) whose life histories have been studied, develop to the infective stage in a mollusk or an annelid. Thus far, in spite of intensive searching at this laboratory, we have been unable to find any evidence that an intermediate host, even a facultative one, is involved in the life cycle of *F. hirthi* (*3*).

Because of *F. hirthi*'s small size (*7*) and widely scattered distribution, isolation of first-stage larvae is tedious and difficult. Therefore, I ground up entire lungs infected with *F. hirthi* and, on 1 August, and again on 15 August 1975, fed them to one of five beagle dogs from an uninfected source. Fifteen other beagle dogs belonging to the same lot had been found free of infection on postmortem examination. On 15 November 1975, I found adult *F. hirthi* male and female worms in the lungs of this dog but none in two controls that I examined at the same time. I now ground up these new infected lungs and fed them to one of the two remaining dogs. A week later I received infected lungs from another source and fed 28 small pieces, each containing at least one gravid female worm, to the same dog. On 20 February 1976, I found *F. hirthi* in the lungs of this dog but none in the control. The infective agents transmitted by feeding lung tissue could only have been the first-stage larvae encased in their eggshells in the uteri of female worms, because the only other life stages that I could demonstrate in this material were pubescent and mature male and female worms. To establish the infectivity of first-stage larvae beyond doubt I completely isolated gravid female worms from lung tissue and administered these by gelatine capsule to two beagle pups. From 10 to 30 worms were thus administered each week for 4 weeks starting on 24 May. On 24 and 25 June, these pups were killed and autopsied and found to be infected with *F. hirthi*, whereas two control pups were found to be free of infection.

I conclude that the infective agents were the first-stage larvae. Thus, autogenous reinfection with *F. hirthi* becomes at least a theoretical possibility. Autogenous reinfection would in turn help to explain the extraordinary apparent longevity of *F. hirthi* infection and the concurrence in the lungs of dogs, both young and old, of living worms surrounded by normal lung tissue and of disintegrating worms surrounded by granulomatous reactions (*3*). In an evolutionary context, this behavior could be viewed as a substitution, by the parasite, of definitive host-tissues for the tissues of an intermediate host as a substrate for the development of larval stages 2 and 3.

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1. I have repeatedly identified *F. hirthi* in beagle lung tissue submitted by two of these establishments. Other workers have reported to me that they have diagnosed this infection in beagle dogs supplied by the other three establishments.
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6. *Probstmayria vivipara* (Oxyurata), *Capillaria phillipinensis* (Trichurata), and the hyperinfective form of *Strongyloides stercoralis* (Rhabditata) are the only nematode parasites of domestic animals and man known to be capable of completing their life histories and thus of multiplying within their host.
7. Male specimens average 2.8 mm in length and 0.038 mm in width; females average 9.9 mm in length and 0.084 in width.

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Antiserum to Brain Gangliosides Produces Recurrent Epileptiform Activity

Abstract. *A single injection of 10 microliters of antiserum to total brain ganglioside onto (and into) the sensorimotor cortex of the rat resulted in recurrent spiking in the cortical electroencephalogram, lasting from 7 to 17 days. Absorption of antibody with pure monosialoganglioside (G_{M1}) completely abolished the effect. Spiking was reactivated after 4 weeks by intramuscular injection of pentylenetetrazole.*

The essential problem in epilepsy is to understand mechanisms underlying discharges which characterize the "hyperphysiological" state at the epileptogenic focus in the cortex (*1*). Experimental models of epilepsy involving tissue damage (freezing, or application of alumina cream or cobalt), pharmacological agents (such as penicillin and strychnine), or electrical stimulation have not provided a clearly defined target for examining mechanisms. In contrast, the immunoneurological model (*2*) provides such a target because of the well-recognized molecular specificity inherent in antigen-antibody reactions. Exploitation

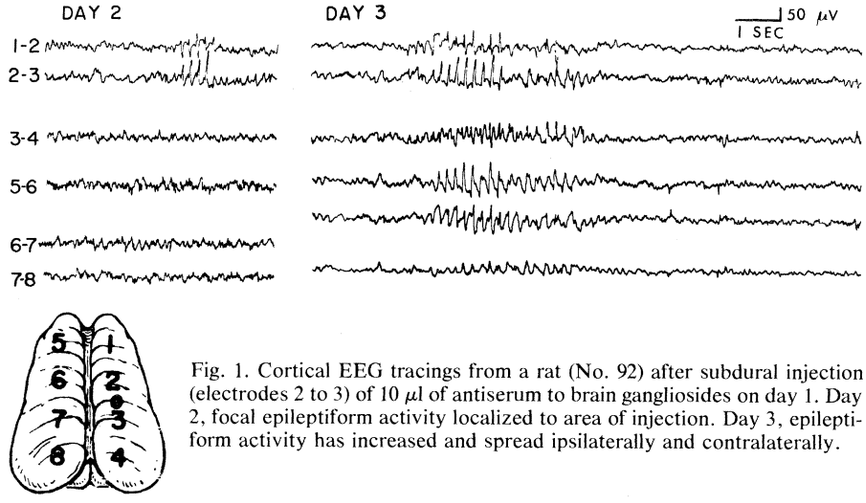

Fig. 1. Cortical EEG tracings from a rat (No. 92) after subdural injection (electrodes 2 to 3) of 10 μl of antiserum to brain gangliosides on day 1. Day 2, focal epileptiform activity localized to area of injection. Day 3, epileptiform activity has increased and spread ipsilaterally and contralaterally.

of the potential of this model, in which seizure activity is produced by administration of specific antibody, has been impeded by the complexity of the antigenic systems used to obtain effective antibody. We have now found a simple, reproducible immunological system that should permit extensive development of this model based upon neurochemical criteria.

We have been investigating the effects of antiserum to the synaptic membrane fraction (*3, 4*), and have confirmed and extended previous observations (*5*) that this agent can cause epileptogenic activity after either intraventricular or subdural injection. The specificity of the effect on the electroencephalogram (EEG) is attested to by the failure to elicit any response with antiserums to other brain components such as the nervous tissue specific proteins S-100 and 14-3-2, or to myelin, or to other membranes such as erythrocyte stroma (*4*).

Since antiserum to the synaptic membrane fraction contains antibodies reacting with brain gangliosides (*6*), and since gangliosides are important components of synaptic membranes (*7, 8*), we have studied the effects of antiserum to brain gangliosides on EEG activity. Antiserums were prepared against the total ganglioside fraction of bovine brain (*9*). As has been previously shown (*10*) these antiserums reacted in vitro with G_{M1} and G_{D1b} ganglioside species, but not with either G_{D1a} or G_{T1}. Three antiserums prepared in different rabbits were used in this study. When these antiserums were absorbed with pure G_{M1} ganglioside (*11*), their capacity to react with ganglioside molecules in vitro was reduced by more than 95 percent (complement fixation).

An array of eight stainless steel cortical screw electrodes was implanted into the calvarium of each rat (Sprague-Dawley, male, ~ 250 g). While under ether anesthesia, the rats were injected, over a 5-minute period, subdurally (*12*) with 10 μl of either whole antiserum to brain gangliosides or such antiserum previously absorbed with pure G_{M1} ganglioside. The animals recovered uneventfully. The EEG recordings were taken for 20-minute periods (i) immediately after injection, (ii) after recovery from anesthesia, and (iii) daily postoperatively.

Initial changes in the EEG were observed 24 hours after the rats were injected with native (untreated) antiserum. At first (day 2) there was focal spiking activity, localized to the site of injection. On subsequent days this epileptiform activity spread both ipsilaterally and contralaterally (Fig. 1), becoming more frequent (3 to 4 bursts per minute) and more sustained (5 to 20 seconds per burst). Twenty rats were tested with the three antiserums against brain gangliosides. Of these, 18 rats developed recurrent epileptiform activity. In 12 rats the EEG abnormalities persisted for 7 to 10 days; in five rats, for 11 to 12 days; and in one rat, for 17 days. As the epileptiform activity receded on successive days, it became less diffuse and more focal. Two of the six rats receiving the highest-titered antiserum showed convulsions, involving the forelimbs and head, on day 3 after injection. The optimal control for both the biological effect and the immunological specificity of the antiserum is absorbed antiserum, the identical reagent from which antibodies are removed specifically by absorption with pure ganglioside. None of the ten animals receiving injections of absorbed antiserum showed any effects. As is indicated above, in previous experiments antiserums to S-100 protein, to 14-3-2 protein, to myelin, or to erythrocyte stroma did not induce seizures (*4*).

Four weeks after the EEG had returned to normal, the abnormal EEG activity could be reactivated with an intramuscular injection of pentylenetetrazole (20 mg/kg). In the seven animals tested, such reactivated spiking activity remained localized to the initial site of antiserum injection and lasted for 1 to 2 hours. This reactivation must be attributed to the original insult since no spiking was seen after pentylenetetrazole injection in rats receiving absorbed antiserum.

Selected rat brains were examined histologically 1 and 14 days after injection (*13*). At the site of injection occasional localized areas of edema were observed. Some sections showed small cellular aggregates characteristic of chronic inflammatory reaction beneath the surface. No areas of necrosis were seen. Since there were no differences between brains of rats receiving native and absorbed antiserum, these reactions must be regarded as nonspecific in nature.

In comparing the effects of antiserum to the synaptic membrane fraction and antiserum to brain gangliosides on EEG activity, two differences were noted. Antiserum to brain gangliosides (i) produced electrical seizure activity having greater frequency and duration and (ii) caused convulsions in several animals. These differences may be accounted for by two factors: antiserums to gangliosides have much higher levels of antibody directed against gangliosides as compared to antiserums to synaptic membranes (as judged by complement fixation titer); and the antibodies in antiserums to synaptic membranes are directed against components of the membrane other than gangliosides. Current experiments are being designed to study these factors and to establish the dose-response curves that will permit sensitive discrimination between different antibody-antigen systems. Components of nerve terminals other than gangliosides may serve as antigenic receptors that can combine with antibodies to alter EEG patterns. Bowen *et al.* (*14*) have produced epileptiform activity in rats with 100 μl of antiserum against a partially purified actomyosin-like protein from rat brain synaptosomes.

Localization of the effect of antiserum to gangliosides (that is, to a single specific antigenic determinant in the outer presynaptic membrane) is suggested by two observations. (i) The effective antibody is completely removed by absorption

with pure G_{M1} ganglioside and (ii) G_{M1} is the only ganglioside species that is labeled when intact synaptosomes are treated first with galactose oxidase and then with ^{3}H-labeled sodium borohydride (*8*).

The potential for specific localization by autoradiography or immunohistological methods is, of course, one of the major advantages of the immunological model. Another is that the absence of gross tissue damage may bring this model close in character to clinical epilepsies of nontraumatic origin. However, the most striking advantage arises from the capacity of immunological methods to provide molecular assignments to reaction sites and thus to indicate whether one or multiple antigenic sites are involved in disruption of synaptic pathways by formation of specific antigen-antibody complexes. As an experimental model of epilepsy, it is important to examine synaptic junctions in various brain regions for evidence of morphological change. Such an examination will have much greater significance when highly purified antibodies are administered rather than whole antiserum.

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9. Gangliosides were prepared from gray matter according to J. N. Kanfer [*Methods Enzymol.*, **14**, 660 (1969)] and used without alkaline hydrolysis. The preparation contained 27.2 percent sialic acid and 0.56 percent phosphorus. The four major ganglioside species, G_{M1}, G_{D1a}, G_{D1b}, and G_{T1} comprised more than 90 percent of this material. Antiserums were prepared in rabbits as described by T. A. Pascal, A. Saifer, J. Gitlin [*Proc. Soc. Exp. Biol. Med.* **121**, 739 (1966)].
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11. The three antiserums to gangliosides used in our study had antibody titers greater than 150 against pure G_{M1} ganglioside in a complement fixation test with 4 units of complement. Absorptions were carried out as follows. To a solution of 25 μg of pure G_{M1} and 50 μg of lecithin in 0.05 ml of ethanol and 0.45 ml of saline, 0.5 ml of antiserum was added. After incubation with stirring at 25°C for 2 hours, the solution was kept at 4°C overnight and then centrifuged at 24,000*g* for 1 hour at 4°C. The upper 0.95 ml of clear supernatant was used for testing. Unabsorbed antiserum was treated in the same way, but without addition of the ganglioside-lecithin mixture.
12. A 25-gage needle held rigidly by a stereotaxic manipulator was inserted to a depth of 1.5 mm below the exposed dura over the sensorimotor cortex. Histological examination showed that deepest penetration of tissue was 0.5 to 1.0 mm.
13. Brains were embedded in paraffin, coronal sections (10 μm) were taken from an area extending 4 mm anterior and 4 mm posterior to the locus of injection, and stained with hematoxylin and eosin.
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Histamine Inhibition of Neutrophil Lysosomal Enzyme Release: An H2 Histamine Receptor Response

Abstract. *Human polymorphonuclear leukocytes treated with cytochalasin B release the lysosomal enzyme beta glucuronidase during contact with serum-activated zymosan particles. Histamine increases intracellular cyclic adenosine monophosphate and inhibits release of this enzyme. The H2 antihistamine metiamide blocks the histamine inhibition of lysosomal enzyme release and the increase in the intracellular adenosine 3,5'-monophosphate of granulocytes. Chlorpheniramine, an H1 antihistamine, did not block the histamine inhibition of granulocyte lysosomal enzyme release.*

Aside from its well-known role as a mediator of anaphylaxis, the physiological importance of histamine remains largely undefined (*1*). Recent evidence suggests that histamine also has an important modulating effect on the function of a variety of cells involved in immunity and inflammation. For example, histamine will inhibit leukocyte chemotaxis (*2*); the release of lysosomal enzymes (*3*); antigen-induced IgE mediated release of histamine from peripheral leukocytes (*4*); the cytolytic activity of effector T lymphocytes in mice (*5*); and the release of macrophage migration inhibitory factor by sensitized lymphocytes (*6*). These last three effects are mediated by H2 histamine receptors. We report here that histamine inhibition of the release of a lysosomal enzyme, β-glucuronidase (E.C. 3.2.1.31), from human neutrophils in the presence of serum-activated zymosan is an H2 receptor response probably mediated through adenosine 3,5'-monophosphate (cyclic AMP).

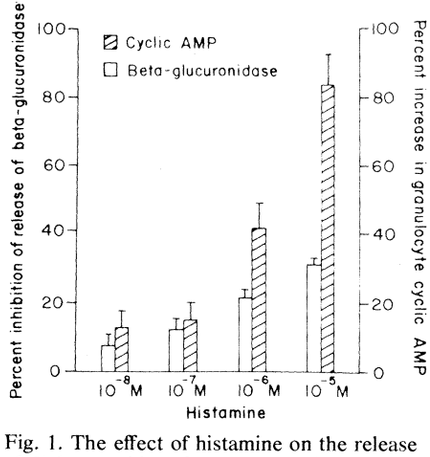

Fig. 1. The effect of histamine on the release of β-glucuronidase from neutrophils exposed to serum and zymosan and changes in the concentration of neutrophil cyclic AMP. Each result represents the mean ± standard error of five separate experiments. Human neutrophils (3×10^6 in 1 ml of HBSS) were incubated for 5 minutes with cytochalasin B (5 μg/ml, final concentration) at 37°C; they were then incubated for 15 minutes with histamine, and then for 30 minutes with zymosan and serum. Control incubations (no histamine) yielded glucuronidase value of 22.2 ± 0.7 (mean ± standard error) μg of phenolphthalein per 18 hours per 10^6 neutrophils which was 32.8 percent of the total enzyme activity. Beta glucuronidase release without serum and zymosan was 2.5 ± 0.5 μg of phenolphthalein per 18 hours per 10^6 neutrophils. Baseline cyclic AMP concentration was 1.25 ± 0.13 picomole per 10^6 neutrophils.

Ash and Schild (*7*) postulated that at least two types of receptors were involved in the histamine response. Responses of the H1 histamine receptor include vasodilatation and smooth muscle contraction which are blocked by antihistamines such as mepyramine, diphenhydramine, and chlorpheniramine. Gastric acid secretion, rat uterine muscle relaxation, and guinea pig atrium contraction follow histamine stimulation of receptors not blocked by the above antihistamines. It was possible to confirm the presence of H2 histamine receptors when the new antagonists, burimamide, metiamide, and cimetidine, were discovered which specifically block the second group of responses to histamine, but not the first (*8*).

Enzymes released from granulocyte