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# Concurrent Infections in mice with *Trypanosoma equiperdum* and *Trichinella spiralis*

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The literature contains numerous reports on concurrent parasitic infections in experimental animals. Reference is here made only to those involving trypanosomes and trichinella worms, the forms used in the experimental results reported here.

Few studies have been reported involving the hemoflagellate *Trypanosoma equiperdum* in concurrent infections. Ee-siriporn and Wagner (1969) reported a synergistic response to an infection with *Schistosoma mansoni* in mice. The mice that were infected with the blood fluke, *S. mansoni* for a duration of 5, 7 and 9 weeks did not survive as long as mice infected for 1 and 3 weeks, or controls infected only with *T. equiperdum*. Rigby and Chobotar (1966) studied the effects of infection with *Trypanosoma lewisi* on the simultaneous development of *Hymenolepis diminuta* in Sprague-Dawley rats. The mean wet weight of *H. diminuta* was significantly less in heavily inoculated ($7\times10^6$ trypanosomes) rats, than that of worms in more lightly inoculated ($1\times10^6$ trypanosomes) rats. Worms in rats which received trypanosomes on the 8th day of the cestode infection weighed significantly less than worms in control rats. The weight of worms in rats receiving trypanosomes on the same day as the cestodes was not significantly different from that of worms in control rats.

*Trichinella spiralis* is known to have two effects on its hosts in concurrent infections; synergistic in some cases and in others, a protective effect. Lubiniecki *et al.* (1974) observed that a suitably-timed pre-existing *T. spiralis* infection greatly enhanced the intracerebral replication and mortality of peripherally-inculated Japanese B encephalitis virus. The increased mortality was associated with a decreased survival time and the effect was maximal when virus challenge occurred 7 days following the helminthic infection. Young adult rats given a combined infection of *T. spiralis* and encephalomyocarditis virus by Kilman and Oliver (1961) experienced a high incidence of crippling and death while control rats given virus alone had a lower incidence of crippling. On the other hand, there was a decreased susceptibility to *Listeria monocytogenes* in mice after infection with *T. spiralis* as reported by Cypess *et al.* (1974), and a similiar protective effect was observed by Larsh and Campbell (1952) with *T. spiralis* and *Hymenolepis nana*.

## Materials and Methods

Three tests were conducted to determine the effects of concurrent infections with the hemoflagellate and the tissue nematode. The mice used in these tests were females of the Swiss Webster albino strain. The parasites were the NIH strain *T. equiperdum* and the *T. spiralis* was obtained from a strain maintained by Dr. L. A. Ash of the University of California at Los Angeles. The trypanosomes were maintained in the frozen state at −60°C to −70°C. The blood was thawed and several mice were given intraperitoneal injections. Three days later blood with a high parasitemia was transferred intraperi-

toneally to Sprague-Dawley-derived rats. These rats esrved as the source of trypanosome infection. The experimental mice were likewise given intraperitoneal injections at the rate of approximately $1.5\times10^5$ trypanosomes in blood diluted with a transfer solution (Chen, *et al.*, 1945) (tests 1 and 2), and in undiluted blood in the amount of 0.1 ml (test 3). The material was maintained at a temperature of approximately 25–35°C. *T. spiralis* larvae were digested from the muscles of the donor rats in the artificial gastric juice containing 6 % pepsin and 7 % HCl. The larvae were suspended in a nutrient broth solution consisting of 10–20 % gelatin and 2 % tryptose in water. Each mouse received by gavage 40 larvae per gram body weight, or approximately 800 larvae per 20 gram mouse.

Each group of mice was given the larvae on the various days designated in the three experiments. For tests I and III this represented 15 different infection dates over a period of 29 days. All of the mice including the controls, were given the injections of trypanosomes during the same period of time on the designated Day 0. The number of mice used for each group for tests I and II was 15, and for test III it was 10.

## Results

The findings of the three tests are graphically portrayed in Figure 1. There is general agreement to show that the mice infected with *Trichinella* were protected to the extent that they survived the concurrent *T. equiperdum* infection significantly longer than the trypanosome control mice. This was especially true for those mice with the worm infection of durations of 1, 3–5 days, and again from days 9 to 29.

In all the tests the mice infected with

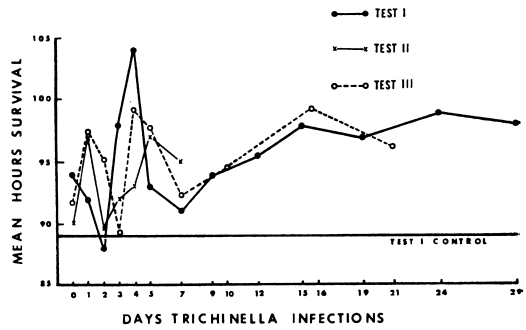


Fig. 1. Survival times in mice infected with *T. equiperdum* controls, and simultaneously with *T. spiralis* at the designated durations of times in days. Three tests are recorded, adjusted to Test I controls

Table 1 Effects of *Trypanosoma equiperdum* on the survival of mice infected with *Trichinella spiralis*

| Days of Worm infect. | Hours of Survival | | | | | |
|---|---|---|---|---|---|---|
| | Range | Mean | S.D. | S.E. | Diff. of means | *t* value |
| 0 | 49–54 | 51.7 | 0.52 | 0.16 | 2.8 | 6.84 |
| 1 | 48–74 | 55.5 | 8.65 | 2.74 | 6.6 | 7.01 |
| 2 | 51–57 | 52.5 | 0.68 | 0.22 | 3.6 | 8.3 |
| 3 | 45–55 | 49.2 | 3.31 | 1.05 | 0.3 | 0.60 |
| 4 | 49–82 | 59.2 | 11.3 | 3.57 | 10.3 | 8.81 |
| 5 | 48–92 | 57.8 | 12.6 | 3.99 | 8.9 | 6.78 |
| 7 | 49–56 | 52.3 | 2.26 | 0.72 | 3.4 | 7.76 |
| 10 | 49–58 | 54.4 | 2.99 | 0.95 | 5.5 | 11.48 |
| 16 | 54–68 | 59.4 | 4.44 | 1.4 | 10.5 | 21.58 |
| 21 | 51–62 | 56.1 | 3.92 | 1.24 | 7.2 | 3.9 |
| Control | 42–56 | 48.9 | 3.76 | 1.19 | | |

*Trichinella* and trypanosomes for 2–3 days died earlier than the controls, or had about the same survival time. Test II was done to check this unusual finding as a result of Test I and therefore covered only the first seven days of worm infection. Table 1 presents the data for Test III in which each group was represented by 10 mice. These results appear in Figure 1 and are compared with tests I and II.

## Discussion

Mice infected with *T. spiralis* for certain durations of time, are protected against otherwise earlier death times when concurrently infected with *T. equiperdum*, which is fatal to mice at a mean death time (Test I) of less than 90 hours. Extended survival times are seen in mice infected for days 1 and 3 to 5, and from day 9 on to day 29, depending on the number of trypanosomes injected. This probably represents a competitive host-parasite relationship between the two forms of parasites. On what basis this competition exists is not determined; nor whether an immunological protective means is provided by the tissue nematode.

In an attempt to explain the distinct change in this pattern, that is, from protective role to synergistic, as seen in day 2 (of Test I) and again in days 5 and 7, where the survival time in the mice was less than, or about the same as the controls for trypanosome-induced deaths. Figure 2 is presented. This is based on results obtained from Test I.

Here are indicated (at the arrows) the major events that occur in the mice as an infection proceeds from the point in time of ingestion of the larvae. Not shown is perhaps a very significant event that takes place on or about day 2. That is the invasion of the mucosa, and subsequent molting of the larvae. This may account for the lowest survival time experienced in the test. Again, at days 5 and 7, gravid females invade the mucosa where larviposition takes place. At these times, survival times in the mice is again at a low point. Thus, a brief

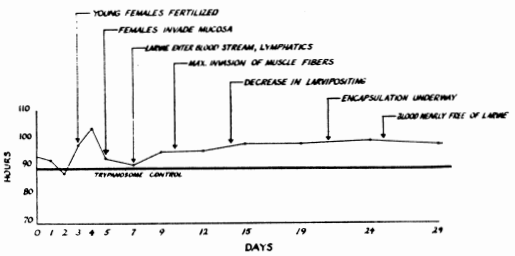


Fig. 2 Events taking place during the course of an infection with *Trichinella spiralis* in the mammalian host. Omitted is the invasion of the intesinal mucosa by the newly ingested larvae and their molting at about days 1 and 2

synergistic effect would result due to intestinal tissue invasion of the worms. At day 4 when a high survival time is reached, the worms are probably in the lumen of the intestine. A competitive, or protective effect is seen when the worms are in the intestinal lumen, and again as the larvae migrate to the striated muscles and become encysted.

## Summary

Mice infected with *T. spiralis* for different periods of time, daily from day 0 through day 5 and at various other periods to day 29, were injected with *T. equipderum*, as were control mice to establish the death times in the mice with the trypanosome infection only. It was noted that the mice with the *Trichinella* infections of approximately 1 and 3 to 5 days, and again from days 9 to 29 survived significantly longer than the control mice; thus a protective effect. Conversely, mice infected for 2 days and for days 5 to 7, died earlier, or at about the same time as the control mice, suggesting a synergistic, or no, effect. It is postulated that the protective effect might be due to an immune response to the trichina-worm infection, which may have inhibited somewhat the multiplication of the trypanosomes, but that the synergistic effect was due to intestinal tissue invasion by the larvae (day 2) and adults (days 5 to 7).

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