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IMMUNITY OF *TRICHINELLA SPIRALIS* IV. PASSIVE IMMUNITY IN MICE

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Abstract. It was found that homologous anti-*Trichinella spiralis* serum caused an immune response in mice infected with the parasite. This was shown by a reduction in the number of larvae encysting in the muscles. Further experiments showed that no reduction in the longevity of the adult worm was produced. No effect upon the muscle larvae themselves was shown and it is suggested that the principle effect of passive transfer of immunity is on the fecundity of the worms.

There are conflicting reports concerning the possibility of passively transferring immunity to *Trichinella spiralis*. SALZER (1916) claimed to have obtained a temporary alleviation of clinical trichinellosis in man by transferring serum from a patient who had recovered from the disease. He also claimed to have shown that serum from immunized rabbits was capable of giving an "almost complete prophylactic response" in other rabbits. SCHWARTZ (1917) and HALL and WIGDOR (1918) both maintained that it was not possible to passively transfer immunity to *T. spiralis* in a variety of experimental animals. CULBERTSON (1942) and GEORGESCU, SIMONESCU and COMAANESCU (1964) were able to transfer immunity in rats with homologous anti-trichinella serum but MCCOY and BOND (1941) could not do so. LARSH, GOULSON and WEATHERLEY (1964a and b) could not obtain passive transfer of immunity in the mouse using homologous serum or whole blood although they only used the number of adult worms in the intestine as a criterion of immunity. Rabbit anti-trichinella serum has also been used to passively immunize rats (TRAWINSKI 1935; OLIVER-GONZALES 1941) and mice (CULBERTSON and KAPLAN 1937 and 1938, MILLS and KENT 1965). CHUTE (1956) found that immune rat serum caused the larvae in the muscles of treated rats to be smaller than normal. In the following experiments homologous immune serum was used in mice with *T. spiralis* infections.

MATERIALS AND METHODS

The method of counting larval and adult *T. spiralis* and of infecting mice have been reported elsewhere (DENHAM 1968). The London strain of *T. spiralis* was used in all the experiments. Randomly bred ICI albino mice were used in experiments 1, 2, 3 and 5 and CFW randomly bred albino mice used in experiment 4. In each experiment the serum was administered by intraperitoneal injection.

EXPERIMENTS AND RESULTS

Experiment 1. In this experiment the effect of immune mouse serum on an infection in normal ICI mice was studied by observing the number of larvae which encysted in the muscles. Serum was collected from the mice used in experiments reported by DENHAM (1966). Thirty-three female mice were infected with 400 *T. spiralis* larvae each. Two days before this infection 15 of these mice were given 0.25 ml of serum and at the time of infection were given 0.5 ml each. Further injections of serum were given to these mice 3 days (0.25 ml each), 10 days (0.5 ml each) and 15 days (0.5 ml each) after infection. On the 35th day after infection all the mice were killed and the number of larvae in their muscles was determined. The control mice contained a mean of 99,000 (SE 7,200) larvae and the infected-serum treated mice 52,600 (SE 2,500) larvae. The difference between the groups is highly significant ($p = > 0.01$).

Experiment 2. Since experiment 1 showed that some immunity was transferable with serum from immune mice an experiment was conducted to determine whether an early elimination of adult *T. spiralis* from the gut of mice could be initiated. The serum was obtained from mice given 3 immunizing infections of 400 larvae each. Twelve female ICI mice were infected with 400 larvae each. Six of these were given 0.5 ml of serum each at the same time as the infection and 0.3 ml each per day for the next 8 days. Two days later all the mice were killed and adult worm counts made on them. The control group had a mean of 165 (SE 16) adults and the group infected and given serum 172 (SE 23) adults. The difference is not statistically significant.

Experiment 3. In this experiment the effect of immune serum on the duration of a low level infection was studied as DENHAM (1968) found that a low level infection of *T. spiralis* in mice (100 larvae per mouse) was much more susceptible to the immune response of the host than were high level infections.

Twenty five female ICI mice were infected with 100 larvae. Ten of these were treated with 0.25 ml serum (from mice given 3 immunizing infections) on the day before infection and 0.2 ml per animal on each of the succeeding 8 days. Five untreated mice were killed 5 days after infection and were found to harbour a mean of 93 (SE = 8.9) adult worms. The other two groups of 10 mice were killed 11 days after infection. The control mice and the group given serum both harboured a mean of 39 worms.

Experiment 4. DENHAM and MARTINEZ (unpublished observations) found that the CFW strain of albino mice responded much more strongly to *T. spiralis* than ICI

mice and in this experiment serum from CFW mice infected twice with 400 *T. spiralis* larvae each was used. Two groups of 5 male CFW mice were infected with 100 larvae. The mice of one group received 0.5 ml of serum on 8 consecutive days starting the day before infection. The mice were killed and adult worms counted 6 days after infection. The normal controls had a mean of 50 worms each and the serum-treated group 49 worms each.

Experiment 5. This experiment was designed to show the effect of immune serum on the parenteral phase of the infection independent of any effect on the adult stage. Thirty-five mice were infected with 400 larvae. Seven days later they were treated with methyridine at 500 mg/kg. This was repeated 3 hours later and the next morning. This treatment schedule was calculated to remove the intestinal worms (DENHAM 1965) and controls showed that this had been done. The remaining 32 mice were divided into 2 groups of 16. One was left untreated and the other treated with 0.5 ml of immune serum 2 days after the last methyridine treatment, 0.25 ml on the next 3 days and 0.25 ml on the 6th, 8th, 10th and 13th days. All the mice were killed and larvae counted seven days after the last serum was injected. The counts showed 21,600 (SE 2,100) in the normal controls and 20,900 (SE 2,500) in the serum treated group. All the larvae collected from each group were mixed together and a sample from each group heated at 70 °C for 10 minutes to straighten them. Fifty larvae from each group were drawn and measured. The control larvae were 1.13 (SE 0.04) mm long and the treated larvae 1.13 (SE 0.03) mm long.

DISCUSSION

Experiment 1 demonstrates that immune mouse serum is able to transfer immunity to recipient mice if immunity is assessed by the number of larvae encysting in the muscles. It appears, however, that this reduction in the number of larvae in the muscles is not due to a reduction in the longevity of the adult worms (LARSH et al. 1964 and b and experiments 2, 3 and 4 of the present paper). COKER (1956) reported that serum from cortisone treated immunized mice was able to reduce the level of adult worms in a *T. spiralis* infection although in the full report of this experiment (COKER 1954) he found that serum from immunized mice which had not been treated with cortisone was not able to transfer this immunity. It is possible, therefore, that this result was due to chance rather than to passive transfer of immunity, especially as the reduction in the adult worm burden was only from 145 to 122 with five mice per group.

As homologous immune serum does not appear to stimulate the early expulsion of an adult population of *T. spiralis* from the mouse it is noteworthy that CULBERTSON and KAPLAN (1937 and 38) and MILLS and KENT (1965) obtained this expression of immunity using immune rabbit serum injected into mice. Unfortunately there does not seem to be any recorded experiment on the extent of the immunity passing from mother to young mice as this would give a good indication as to whether

immunity can be transferred by means of homologous globulins. It has been shown that the female hamster, rat and rabbit do pass immunity against *T. spiralis* to their young (MAUSS 1940 and CULBERTSON 1943).

In experiment 5 it was found that neither the number of worms encysting nor their size was affected by immune serum given to the animals after the elimination of adult worms by methyridine. CHUTE (1956) found no difference in the number of larvae encysting in rats treated with anti-*Trichinella* serum from rabbits when treated between the 16th and 24th or 20th and 26th days after infection. He did however, find that larvae from these rats were 1.03 (SE 0.039) mm long whereas those from rats given normal rabbit serum were 1.11 (SE 0.023) mm long. It is remarkable that serum should have such an effect so long after the birth and migration of the larvae in rats as those rats treated with immune serum from 20—26 days after infection presumably contained very advanced larvae at this time. It has been shown that actively immunized mice harbour smaller larvae (1.08 SE 0.01 mm) than do control mice (1.15 SE 0.01 mm) (DENHAM unpublished observations).

As neither the longevity of the adult worm (experiments 2—4) nor the maturation of the new generation of larvae (experiment 5) is affected by immune serum and yet the number of larvae finally encysting is reduced after treatment with serum (experiment 1) it is highly likely that serum transfer primarily affects the fecundity of the adult worms.

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WHO INTER-REGIONAL COURSE ON METHODS OF EPIDEMIOLOGICAL SURVEILLANCE IN KARLOVY VARY (CZECHOSLOVAKIA)

Between 12—31 August 1968 the World Health Organization held an Inter-Regional Course on Methods of Epidemiological Surveillance in Karlovy Vary, Czechoslovakia, with participation of prominent scientists and responsible officers of the WHO Regional Offices, as well as medical workers of key countries for WHO programmes (Ghana, Mexico, Chile, Venezuela, Iran, East Pakistan, UAR, Netherlands, Hungary, India, Ceylon, Japan). The Organizing Secretary was Docent Dr. K. Žáček.

The Course was opened by Dr. Karel Raška, Director, Division of Communicable Diseases, WHO, Geneva, with an important paper on the concept of epidemiological surveillance of communicable diseases. Dr. Raška defined epidemiological surveillance as follows: "Surveillance means the epidemiological study of a disease as a dynamic process involving the ecology of the infectious agent, the host, the reservoirs and the vectors, as well as the complex mechanisms concerned in the spread of infection and the extent to which this spread occurs. Surveillance provides a scientific basis for public health decisions on control programmes, their evaluation and for epidemiological forecasts." — "The aim of surveillance is to follow up specific diseases in terms of morbidity and mortality in time and place and to follow the spread of infection in the human population (i.e. circulation of the etiological agent, immune response) and, in certain diseases, such as salmonellosis, plague, tularemia, brucellosis, Q fever, arbovirus infections, toxoplasmosis etc., among the animal population also. Diagnosis, and especially tracing the spread of infection, may involve a variety of laboratory procedures."

Dr. Erik Roelsgaard, Chief Medical Officer, Epidemiological Surveillance Unit, WHO, Geneva, dealt with elements of epidemiological surveillance and their implementation under different conditions, which, according to the report of the Technical Discussions at the Twenty-first World Health Assembly, are as follows: