## *Trichinella spiralis:* Acceleration and inhibition of cyst calcification in rats

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*Summary.* Daily administration of vitamin $D_3$ (75,000 IU/kg b.wt) for 7 days accelerated *Trichinella spiralis* cyst calcification in rats with a 14-week-old infection. When disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP) was administered (50 mg/kg b.wt) from 2 days before until 2 days after vitamin $D_3$ treatment, cyst calcification was inhibited. Thus, the ability to inhibit *T. spiralis* calcification has been demonstrated for the first time.

A characteristic event in the life cycle of *Trichinella spiralis* is the calcification of the larval cyst in host skeletal muscle. Information concerning calcification of *T. spiralis* cysts has been summarized by Gould[4] as follows: 'Apparently nothing is known of the mechanism of the calcification of trichinella cysts; but the parallel acceleration of calcification of host tissues and of trichinella cysts after administration of ergosterol or parathormone suggests that the same general mechanism prevails.'

Because cyst calcification is characteristic of the *T. spiralis* life cycle, the ability to block this process would be of value in elucidating the importance of cyst calcification in this host-parasite relationship. The ability to block the calcification mechanism could also be of therapeutic value. For example, mebendazole has proved effective against the encapsulated phase of *T. spiralis*[5]. However, after drug therapy, the larva itself is subject to calcification. Granuloma formation occurs around the calcified remnants and the removal of these remnants is a process of long duration[6]. A compound capable of blocking this calcification could thus be of value in leading to a more rapid recovery from a trichinella infection.

Because cyst calcification is a rather slow process, the ability to inhibit the calcification process could be assessed more readily by attempting to block the process after it had been accelerated by drug therapy. According to van Someren[7], previous authors have demonstrated the ability to accelerate and intensify trichinella cyst calcification by administration of parathormone and ergosterol. In our laboratory, vitamin $D_3$ has been used to accelerate cyst calcification.

Diphosphonate compounds have been developed which are capable of preventing calcification in vivo, even after massive doses of vitamin $D_3$. One such compound particularly effective in preventing calcification when administered s.c. or p.O. is disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP)[7].

*Materials and methods.* The strain of *T. spiralis* used in this investigation was obtained from the Department of Parasitology and Laboratory Practice, University of North Carolina at Chapel Hill, USA. Larvae for infection were recovered by digestion. 90-day-old male Holtzman rats were divided into 5 groups (10 rats/group) and infected with 600±100 larvae by gavage. Vitamin $D_3$ (cholecalciferal) was administered (75,000 IU/kg b.wt) daily for 7 days by gavage to rats with 4- and 14-week-old infections. EHDP was obtained from the Proctor and Gamble Co., Cincinnati, Ohio (USA). EHDP was dissolved in distilled water and administered by gavage and s.c. (50 mg/kg b.wt) from 2 days before until 2 days after vitamin $D_3$ treatment. At the end of EHDP administration, the diaphragms from experimental rats were excised and subjected to microscop-

Relative amounts of calcium in whole mounts of *Trichinella spiralis* cysts from rat diaphragms

| Treatment groups (10 rats/group) | Relative amount of calcium deposition |
|---|---|
| Vitamin $D_3$ (14-week infection) | Marked* |
| Vitamin $D_3$ (4-week infection) | Slight** |
| Vitamin $D_3$ and EHDP (oral) (14-week infection) | None*** |
| Vitamin $D_3$ and EHDP (s.c.) (14-week infection) | None |
| Control (14-week infection) | None |

* Marked calcification as was evidenced by calcium deposition extending from pole to pole and encompassing much of the cyst. ** In the 4-week group only slight calcium deposition was observed at the poles. *** No calcium deposition was observed.

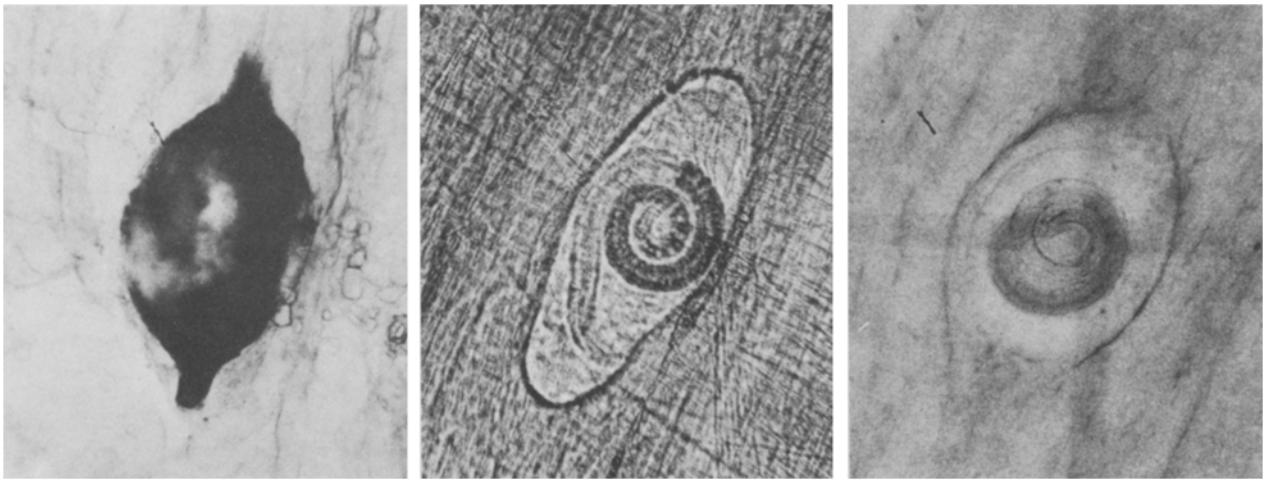

Fig. 1. Marked cyst calcification after administration of vitamin $D_3$ in a 14-week *T. spiralis* infection. Fig. 2. Normal cyst in a 14-week *T. spiralis* infection from a control rat. Fig. 3. The effect of EHDP on a 14-week *T. spiralis* infection from a rat given vitamin $D_3$. ×45.

ic observation and histochemical straining via Von Kossa's method[9] to demonstrate calcium deposition.

*Results and discussion.* Based on histochemical straining and observation of whole mounts, marked calcium deposition in *T. spiralis* cysts was observed in rats with a 14-week infection (figure 1) as opposed to infected control rats not administered vitamin $D_3$ (figure 2). The enhancement of cyst calcification was very slight in rats with a 4-week-old infection.

The main scope of this investigation was an attempt to inhibit the calcification process of the *T. spiralis* cyst. Although a somewhat artifical system was employed in that vitamin $D_3$ was used to accelerate cyst calcification, the end result of vitamin $D_3$ treatment was similar to an infection of long duration. In rats given EHDP while under vitamin $D_3$ treatment, cyst calcification was not evident (figure 3) and the cysts appeared similar to infected rats not receiving vitamin $D_3$ (figure 2). The table lists the relative amount of calcium deposition in the experimental groups. Thus, the ability of EHDP to block the calcification of *T. spiralis* cysts demonstrated for the first time the inhibition of this mechanism. This demonstrates that cyst calcification is not an irreversible process and is subject to drug therapy.

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3 Much appreciation goes to Dr Gordon S. Hassings and the Proctor and Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio USA for providing the EHDP used in this study.
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## Anti-myosin stains chromaffin cells[1]

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*Summary.* The presence in fixed chromaffin cells of antigenic sites for a myosin antibody was demonstrated using immunofluorescence techniques. Tests on viable cells showed that at least some of the antigenic sites seem to be localized on or close to the cell surface and explained the cell agglutination that occurred with the addition of the myosin antibody to cells isolated by a method described in this paper.

Actin, myosin, tropomyosin and troponin have been isolated from a variety of nonmuscle cells[2,3]. We have recently isolated from the chromaffin cell a protein with electrophoretic mobility, enzymatic activity, amino acid composition and electron microscopic appearance closely resembling those myosins from muscle and nonmuscle cells[4]. Although all major vessels were dissected out of the intact adrenal medulla before myosin was extracted, it remained possible that adrenal myosin originated in the smooth muscle of the small vessels of the medulla rather than in the chromaffin cells. However, myosin has been identified in extracts from cultures of sympathetic neurons[5]; such neurons have the same embryological origin as the chromaffin cells. To determine more directly whether myosin is a component of the chromaffin cells, the present experiments used immunohistochemical techniques to demonstrate the presence in the chromaffin cell of antigenic sites for a myosin antibody.

*Material and methods.* Bovine muscle myosin was prepared and its purity established by polyacrylamide gel electrophoresis as described previously[4]. Rabbit antiserum was prepared against purified myosin, following a procedure previously published[6], and the IgG fraction of this antiserum was isolated as described elsewhere[7]. A normal IgG fraction was also prepared from serum of pre-immunized rabbits. The anti-muscle myosin (AMM) IgG fraction when tested in an Ochterlony plate against either purified bovine muscle myosin or adrenal actomyosin[4,8] gave single precipitin lines which fused completely demonstrating immunological identity (figure 1). No precipitin lines were observed when the antibody was tested against 4 different concentrations of bovine muscle actin (figure 1). Furthermore, the AMM-IgG fraction when tested against bovine aortic muscle myosin gave a precipitin line which fused completely with that obtained against adrenal actomyosin. The AMM-IgG was then tested on isolated chromaffin cells by the standard (indirect) immunofluorescence test[9]. The isolated cells were prepared as follows: a bovine adrenal gland obtained from a slaughterhouse was freed from its cortex and perfused in vitro for 30 min (37 °C) with $Ca^{2+}$-free, $Mg^{2+}$-free Locke's solution as described previously[10]. Perfusion was then continued for 40 min with fresh solution to which 0.05% collagenase (Sigma Chemical Co.) had been

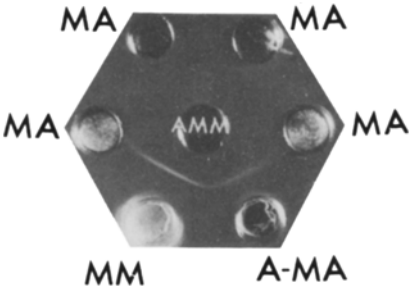


Fig. 1. Double immunodiffusion test between AMM-IgG (center well, 5 µg) against bovine adrenal medullary actomyosin (A-MA, 40 µg), bovine muscle myosin (MM, 40 µg) and 4 concentrations of bovine muscle actin (MA, 160, 80, 40 and 20 µg from left to right). Muscle actin was isolated as described elsewhere[26]. The capacity of each well was 30 µl and the plate contained the following mixture: 1% agar, 0.1% sodium azide, 0.4 M KCl, 0.04 M sodium pyrophosphate and 0.5 M Tris-HCl buffer, pH 8.0. The plate was left at room temperature for 48 h before the photograph was taken.