

## Growth of *Trichinella spiralis* (Nematoda) Muscle Larvae in the Rat

The life cycle of *Trichinella spiralis* and its many parameters in the laboratory rat has been known for many years and comprehensively reviewed<sup>1,2</sup>. There, however, have been only a few reports<sup>3-6</sup> on daily growth of the larvae in skeletal muscle; indicating an incompleteness of life cycle information. Nevertheless, this growth is often and importantly used as a criterion in the efficacy of *T. spiralis* antihelminthics<sup>7</sup>. The aim, therefore, of this paper is to investigate, describe and establish definite parameters concerning this growth (in vivo) in rat skeletal muscle.

**Materials and methods.** Sprague-Dawley male albino rats were used in the investigation. They were maintained individually in polycarbonate cages containing treated bedding. Purina laboratory chow and fresh water were provided ad libitum. At the age of 42 days, rats were inoculated with 3000 infective larvae by intubation. The sample size was 18 rats/day postinoculation (pi). At the appropriate time interval pi, rats were killed with ether fumes and their diaphragms excised. The muscle fibres were teased apart onto microscope slides. Measurements,

in  $\mu\text{m}$ , were made at  $\times 450$  using a micromanipulator and ocular micrometer. 180 measurements/day pi were made (10/rat) on the largest larvae present. Only larval lengths were considered since nematodes show a more pronounced increase in length while growing than they do in width (LEE<sup>8</sup>).

**Results and discussion.** From a comparative point of view, it is apparent that differences in daily mean lengths of *T. spiralis* muscle larvae have been reported in the literature (Table). Since the authors<sup>3-6</sup> have not stated either their sample size, standard error, or standard deviation, statistical comparisons cannot be made against this present study.

From data obtained in the present study, it is evident that a standard growth rate is not present for *T. spiralis* in rat skeletal muscle. The rate of daily growth varies per day pi; is not the same on each successive day pi; and is not logarithmic. The growth rate of this nematode in rat skeletal muscle, therefore, is a discontinuous process.

This indicates that in antihelminthic studies, identical control animals must be used concurrently under identical situations, and that workers cannot depend on the literature for comparisons of larval lengths. Each experiment, where growth is a factor, must be treated separately and on an exact day pi basis.

**Résumé.** Dans le présent travail, on a constaté que le taux d'accroissement de *Trichinella spiralis* dans le muscle strié du rat est très variable et offre un processus discontinu.

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Growth in  $\mu\text{m}$  of *Trichinella spiralis* muscle larvae as reported by various authors

Day pi	Authors and host				
	HARLEY <sup>a</sup> (rats)	ALI KHAN <sup>3</sup> (mice)	THOMAS <sup>4</sup> (mice)	RICHELS <sup>5</sup> (mice)	HEMMERT <sup>6</sup> (rats)
4	— <sup>b</sup>	—	—	—	—
5	107 $\pm$ 10 <sup>c</sup>	—	—	—	100
6	106 $\pm$ 12	—	129	—	—
7	111 $\pm$ 11	140	129	107	137
8	117 $\pm$ 18	163	137	121	—
9	126 $\pm$ 20	175	143	135	—
10	147 $\pm$ 28	205	161	145	158
11	167 $\pm$ 31	225	206	162	—
12	203 $\pm$ 51	270	263	226	—
13	243 $\pm$ 59	347	319	313	255
14	385 $\pm$ 73	404	392	326	—
15	500 $\pm$ 75	525	480	450	327
16	572 $\pm$ 80	705	608	552	—
17	644 $\pm$ 84	850	673	569	601
18	690 $\pm$ 85	—	762	639	—
19	706 $\pm$ 88	—	872	789	862
20	721 $\pm$ 90	—	968	938	—
21	844 $\pm$ 92	—	1075	942	—
22	908 $\pm$ 93	—	1141	1043	—
23	910 $\pm$ 94	—	1160	1079	—
24	921 $\pm$ 94	—	1175	1125	—
25	930 $\pm$ 95	—	1185	1225	—
30	930 $\pm$ 90	920	1217	—	—

<sup>a</sup> Present study. <sup>b</sup> No data given. <sup>c</sup> Mean  $\pm$  S.E.; where  $N = 180$ .

<sup>1</sup> S. E. GOULD, *Trichinosis* (Ch. C. Thomas, Springfield Ill. 1945), p. 1-356.

<sup>2</sup> J. LARSH, *Advances in Parasitology* (Academic Press, N.Y. 1963), vol. 1, p. 213.

<sup>3</sup> Z. ALI KHAN, *J. Parasit.* 52, 248 (1966).

<sup>4</sup> V. H. THOMAS, *Z. Tropenmed. Parasit.* 16, 148 (1965).

<sup>5</sup> I. RICHEL, *Zentbl. Bakt. ParasitKde* 163, 46 (1955).

<sup>6</sup> A. HEMMERT-HALSWICK and G. BUGGE, *Erg. allg. Path. path. Anat.* 28, 313 (1934).

<sup>7</sup> V. GALLICCHIO, *Doctoral Dissertation* (University of Illinois, Ill. 1956), p. 1-89.

<sup>8</sup> D. L. LEE, *The Physiology of Nematodes* (W. H. Freeman, San Francisco 1965), p. 93.

## Inhibition of L-Alanine-induced Germination of *Bacillus cereus* Spores by Theophylline

In a previous study we noticed that theophylline inhibits nucleoside-induced germination of *Bacillus cereus* spores<sup>1</sup>. It will be shown in this report that also L-alanine-dependent germination of *B. cereus* spores is strongly affected by theophylline. Studies on this inhibition may contribute to a better understanding of the mechanism by which L-alanine initiates the sequence of biochemical events leading to germination. As emphasized recently by GOULD<sup>2</sup>, no convincing explanation of the role(s) played by L-alanine in this phenomenon has been so far presented.

**Methods.** 1-week-old spores of *B. cereus*, strain 'R', obtained as described previously<sup>1</sup>, were used throughout this study. In a 'standard procedure', the germination mixture consisted of 0.033 M sodium (Na/Na<sub>2</sub>) phosphate buffer, pH 6.4, L-alanine 2 mM and heat-activated spores (75°C, 15 min in H<sub>2</sub>O) at a concentration of 4.5  $\times 10^7$  to 5.0  $\times 10^7$ /ml. Unless otherwise specified, the experiments were carried out at 34°C. Theophylline (1,3-dimethylxanthine, pure cryst.) was obtained from Merck (Darmstadt, Germany). Germination was followed by decrease in