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[1,2]. There, however, have been only a few reports[3–6] 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* antihelmenthics[7]. 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. Spraque-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 μm, were made at ×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[8]).

Growth in μm of *Trichinella spiralis* muscle larvae as reported by various authors

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

[a] Present study. [b] No data given. [c] Mean ± S.E.; where $N = 180$.

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[3–6] 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 antihelmenthic 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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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[1]. 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[2], 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[1], were used throughout this study. In a 'standard procedure', the germination mixture consisted of 0.033 *M* sodium (Na/Na_2) phosphate buffer, pH 6,4, L-alanine 2 m*M* and heat-activated spores (75 °C, 15 min in H_2O) at a concentration of 4.5×10^7 to 5.0×10^7/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

optical density (OD) of the germination mixture, at 625 nm, in a spectrophotometer Hilger-Gilford. As 'indicators' of the kinetics of germination, the following parameters have been used: 1. the 'extent of germination' (E%), calculated as percentage of OD decrease from OD_i (initial value) to OD_f (final value) of the germination mixture by the formula $(OD_i\text{-}OD_f/OD_i) \times 100$; 2. the 'rate of germination' (V), expressed as maximum percentage of OD decrease per min and calculated graphically from plots of percentage of OD decrease versus time.

Results. Theophylline has been found to affect both the extent and the rate of germination induced by L-alanine in *B. cereus* spores. At a fixed L-alanine concentration, the percentage of inhibition is dependent on theophylline dose, on pH of the germination mixture and temperature. The dependence is shown in Table I and Figures 1 and 2. Under the most favorable conditions of pH and temperature (pH 6,4; 34 °C), maximum inhibition is achieved at a concentration of theophylline of 6 m*M*; at higher concentrations, the inhibition is not increased and it does not exceed the values of 70–75% inhibition of the extent, and 80–85% inhibition of the rate, of germination.

Some structural analogues of theophylline (adenine, guanine, hypoxanthine, xanthine, 1-methyl-xanthine and 1,3,7-trimethylxanthine or caffeine) have been tested for their ability to inhibit L-alanine-induced germination. Only 1-methyl-xanthine and caffeine have shown a significant inhibitory effect. At a concentration of 6 m*M*, 1-methyl-xanthine caused approximately 10% inhibition of the rate of germination without influencing its extent, while caffeine inhibited strongly both the rate and the extent of germination (62% and 53% of inhibition, respectively). Therefore caffeine can effectively replace theophylline as inhibitor of the germination by L-alanine. Moreover, the results indicate that the presence of methyl groups on the xanthine ring is required for the inhibition.

The reversibility of the inhibition provoked by theophylline on germination is demonstrated by the following experiment. Spores were treated with 10 m*M* theophylline in sodium phosphate buffer 0.033 *M*, pH 6,4 for 30 min at 34 °C. After accurate washing out of the inhibitor and subsequent exposure to 2 m*M* L-alanine, these spores germinated as well as untreated ones. Similar results were obtained when the spores were treated with theophylline during the heat-activation.

Spores of *B. cereus* 'R' germinated extensively in response to inosine alone; however, the addition of a small amount of L-alanine (3,0–6,0 μ*M*) to the germination mixture containing inosine resulted in a strong enhancement of the rate of germination. When this enhancement was tested for sensitivity to theophylline, inhibition was not observed (Table II). Therefore, the stimulating effect of L-alanine on inosine-dependent germination is unaffected by theophylline which, in this regard, seems to act as D-alanine[3].

Discussion. We have shown that theophylline is a strong but reversible inhibitor of the germination induced by L-alanine in *B. cereus* spores. As far as the mechanism of

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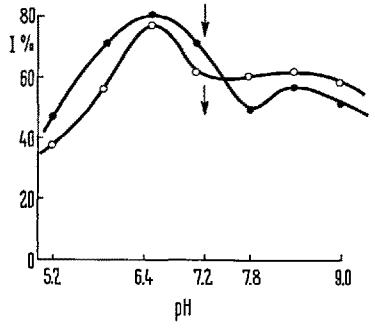

Fig. 1. Effect of pH on the inhibition of L-alanine-induced germination by theophylline. Heat-activated spores (75 °C, 15 min) were incubated either in L-alanine 1 m*M* or in L-alanine 1 m*M* plus theophylline 4 m*M*, at different pH values of sodium (Na/Na_2) phosphate buffer 0.033 *M*. To adjust the pH of the buffer to 5.2 a solution 0.5 *N* of HCl was used. The percentage inhibition (I%) on the rate (—●——●—) and on the extent (—○——○—) of germination, has been calculated as described in Table I. The arrows indicate optimal pH value of the germination in the absence of theophylline.

Table I. Inhibition of L-alanine-induced germination by theophylline

Theophylline concentration (m*M*)	Extent of germination (E%)	I%	Rate of germination (V)	I%
0.0	42.0	–	3.80	–
0.5	42.2	0.0	3.75	1.3
1.0	34.3	18.3	2.80	26.3
2.0	25.5	39.3	1.75	54.0
5.0	15.0	64.3	1.20	68.4
6.0	13.0	69.0	0.80	78.9
10.0	14.0	66.6	0.80	78.9

Heat-activated spores (75 °C, 15 min) were incubated at 34 °C in sodium (Na/Na_2) phosphate buffer 0.033 *M*, pH 6.4 containing 2 m*M* L-alanine and varying concentrations of theophylline, as indicated. The symbol I% represents the percentage inhibition of the germination calculated using the formula $(N_c\text{-}N_t/N_c) \times 100$, where N_c is the value of the extent (or the rate) of germination in absence of theophylline and N_t is the respective value in presence of theophylline. The parameters E% and V are expressed as defined in methods.

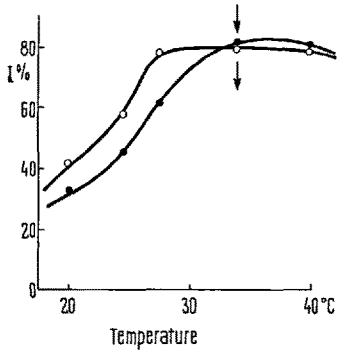

Fig. 2. Effect of temperature on the inhibition of L-alanine-induced germination by theophylline. Heat-activated spores (75 °C, 15 min) were incubated in sodium (Na/Na_2) phosphate buffer 0.033 *M*, pH 6.4 containing 1 m*M* L-alanine without or with 4 m*M* theophylline, at different temperatures. The percentage inhibition (I%) on the rate (—●——●—) and on the extent (—○——○—) of germination, has been calculated as described in Table I. The arrows indicate optimal temperature of the germination in absence of theophylline.

the inhibition is concerned, our data are consistent with the hypothesis that theophylline inhibits some enzymic step involved in germination by L-alanine. A number of enzymes, in particular phosphorylases[4,5] and phosphodiesterases[6], are known to be affected by theophylline; however, there is so far no evidence that the germinative activity of L-alanine depends specifically on one of these enzymes. In *B. cereus* 'R' spores, theophylline inhibits the ribonucleosidase[1] but this enzyme, whose involvement in the germination by nucleosides has been questioned[1,7,8], has presumably no role in germination by L-alanine. Therefore, further studies must be carried out in order to define the specific target of the inhibitory activity of theophylline on germination.

WARREN and GOULD[3] suggested that, in *B. cereus* spores, the role played by L-alanine alone in 'triggering' germination is different from that played by this amino-acid in 'stimulating' nucleoside-induced germination. The fact that theophylline strongly inhibits the germination by L-alanine alone but does not affect the stimulation by L-alanine of inosine-induced germination, gives additional evidence in favour of the suggestion made by WARREN and GOULD.

Table II. Effect of theophylline on the stimulation by L-alanine of inosine-dependent germination

Effectors of germination	Extent of germination (E%)		Rate of germination (V)	
	−	+	−	+
Inosine	40.0	39.2	2.70	2.55
Inosine + L-alanine 3 μM	45.0	44.8	4.80	5.00
Inosine + L-alanine 6 μM	45.0	45.0	5.30	5.25
L-alanine 6 μM	0.0	–	0.0	–

Heat-activated spores (75 °C, 15 min) were incubated at 34 °C in sodium (Na/Na_2) phosphate buffer 0.033 M, pH 6.4 containing the effectors of germination cited in the Table, in the absence (–) and in the presence (+) of 10 mM theophylline. Inosine was used at a concentration of 0.1 mM. The parameters E% and V are expressed as defined in methods.

Riassunto. In spore di *Bacillus cereus*, la teofillina inibisce reversibilmente la germinazione da L-alanina ma non l'attivazione che la L-alanina esercita sulla germinazione da inosina. Il possibile significato di questi dati è discusso.

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THEORIA

Some General Criteria for the Energetical Characterization of Excitable Systems

All living organisms exhibit, at their respective levels of specialization, excitability, allowing them to function suitably in the medium with which they interact. From the physical view point, excitability represents the ability of the system to respond to a certain modification of the *intensive parameters* of the medium by a sudden release of energy, which consequently reduces its free internal energy. Man-made models of biological excitable systems[1] and some other technical devices also have this property.

In this preliminary note, I propose some general criteria for a phenomenological characterization of the functioning of the excitable systems, irrespective of their concrete nature. A more detailed analysis in terms of theoretical physics will follow.

Firstly, we have to point out that all excitable systems are *dissipative structures*[2] which are maintained only by a well-defined cooperation between the internal processes and the fluxes of energy and matter from the external world. In its resting state, the excitable system as a whole possesses a higher potential energy, due to the fact that some of its microscopical components (elementary particles and/or atoms and molecules) are on higher energy levels and prevented by the structure of the system from leaving these levels. If a number of microscopical components pass, however, to lower energy states, this represents 'leakages' which, in terms of non-equilibrium thermodynamics[3], will be denoted by the fluxes J_k^0. The fact that some microscopical components are prevented from reaching the lower energetical levels means that there are in the system generalized (thermodynamical) forces X_k which are maintained constant, despite the leakage fluxes:

$$X_k = cts = X_k{}^o \neq O.$$

This implies that, during the resting state, an energy:

$$\frac{dU_R}{dt} = \sum_k J_k^0 X_k^0$$

is dissipated per time unit. This formula represents the most general expression for the 'resting metabolism' intensity of the excitable systems.

The stimulus changes the structure of the excitable system, so that the forces X_k will be reduced from their resting values X_k^0 to some lower values X_k'. If the system acts according to the 'all or none' law, the forces will vanish, so that an overall energy:

$$U_A = \int_0^\tau \{\sum_k J_k(t) \cdot X_k(t)\}\, dt$$

will be released. The time interval τ represents the refractory period given by the condition:

$$\sum_k X_k(\tau) = 0.$$

The excitable system obeys the 'all or none' law when the stimulus produces a high *cooperative* change of the structure, which means that a local change is propagated by a resonance mechanism, so that there is no equivalence