[Jpn. J. Parasitol., Vol. 42, No. 2, 128–129, April, 1993]

Research Note

Effects of Pepsin-HCl Digestion on the Infectivity of *Trichinella spiralis* Muscle Larvae

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(Accepted for publication; February 17, 1993)

Key words: *Trichinella spiralis,* adult worm, pepsin-HCl digestion

For the mass isolation of larvae of *Trichinella spiralis,* it has been a standard laboratory technique to digest host muscle tissues by an artificial gastric juice. This pepsin-HCl digestion technique is considered to mimic *in vivo* excystation. In fact, larvae thus obtained repeat coiling and uncoiling movements, are importantly infective when administered orally to a new host, and can be used for a wide variety of experimentation.

A question, however, arose whether the pepsin-HCl digestion causes harm to the larvae. When an attempt is made to collect adult worms from intestines of the hosts that were inoculated with the artificially-excysted-larvae, worm recovery is unbelievably low. Rarely is the entire dose of infective larvae accounted for adult worms even in the most permissive host (Despommier, 1983). Interestingly, Stewart and Deford (1989) reported that the infectivity of *T. pseudospiralis* infective L_1 larvae isolated from homogenized muscle in Hanks' balanced salt solution was 3.9 times greater than that for larvae recovered from homogenized carcasses by pepsin-HCl digestion. These together led us to perform a systematic analysis of the effect of pepsin-HCl digestion on the adult worm recovery from host intestines.

Forty female inbred mice (BALB/c aged 6 weeks, purchased from Shizuoka Experimental Laboratory, Shizuoka, Japan) were used. A Polish strain of *T. spiralis* (originally donated from Emeritus Prof. T. Yamaguchi, Hirosaki University School of Medicine) was maintained in ICR mice. Infected mouse muscles were mechanically minced and 0.5g of muscle tissue, conditioned to contain 300 larvae, was given to fasted BALB/c mice (n=5, tartar group). We found that mice eat the tartar meat immediately when it is seasoned with a 0.25M (isotonic) sucrose solution. On the other hand, an aliquot of the muscle tissue was digested by pepsin-HCl solution, composed of 7ml of concentrated HCl, 5g of pepsin (purchased from Nacalai Tesque Co Ltd., Kyoto, Japan) and 1000 ml of tap water, for 1, 2 and 3 hours (1, 2 and 3 hour digestion group, respectively) at 37°C. Three hundred larvae were subsequently administered to the equivalent mice by esophageal pertubation (n=5 for each group). Six days after the innoculation, the mice were sacrificed, the intestine was cut open in the phosphate buffered saline (pH 7.4) at 37°C, and adult worms were collected and counted under a light microscopical observation. It is preferable to fast mice for 24 hours before

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This work was partially supported by a Grant-in-Aid for Scientific Research (No. 01770261) from the Ministry of Education, Science and Culture, Japan.

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Table 1 Comparison of recovery numbers of *T. spiralis* adults and muscle larvae from mice infected with two different sources

Infection source		No. of adults recovered* Mean ± SE	No. of larvae recovered† Mean ± SE
Tartar meat containing L_1		267 ± 14	2925 ± 309
Excysted L_1	1 hr	135 ± 10	1154 ± 219
	2 hr	146 ± 9	1631 ± 206
	3 hr	131 ± 17	1623 ± 201

*Day 6 after infection. †Day 30 after infection.
Excysted L_1 larvae obtained by digestion for 1–3 hour, respectively.
Mean: 5 time experiments. SE: Standard error

sacrificing because it results in easier isolation of worms without intestinal contents. Thirty days after the innoculation the rest of the mice were sacrificed and the number of muscle larvae (per one gram of muscle tissue) were examined by digesting muscles with pepsin-HCl according to the established manner.

Recovered adult worms were 267 ± 14 (Mean ± SE) in the tartar group, 135 ± 10 in the 1 hour digestion group, 146 ± 9 in the 2 hour digestion group and 131 ± 17 in the 3 hour digestion group. There was a significant difference among the tartar group and three digestion groups ($p<0.001$) by the Student's t-test. The same tendency was confirmed for the number of muscle larvae; 2925 ± 309 in the tartar group, 1154 ± 219 in the 1 hour digestion group, 1631 ± 206 in the 2 hour digestion group and 1623 ± 201 in the 3 hour digestion group (Table 1).

Thus the best recovery of adult worms was obtained by administrating encysted larvae. The present data clearly demonstrate that pepsin-HCl digestion causes severe damage to muscle larvae, although the mode of action of pepsin-HCl on larval infectivity is little known except for the reports by Despommier (1983).

Interestingly, even 3 hour digestion does not kill muscle larvae, however, approximately 56% of the worms are injured in such a way that the worms can not parasitize in the host intestine. Only 44% of the worms seem to be responsible for the establishment of new infection. Although the pepsin-HCl digestion method (Larsh and Kent, 1948) is classical and widespread, harmful effect of pepsin-HCl digestion has not been paid much attention. Those effects are also crucial for the statistical study of female fecundity. There are some earlier reports about female fecundity but no general agreement as to the basic question of how many offsprings are produced per one female worm (see review by Despommier, 1983). If such estimation is based on the number of muscle larvae which have been liberated by an artificial digestion, the results apparently would be unreliable. In conclusion, our recommendation for collecting adult worms of *T. spiralis* is that the innoculation should be performed by administrating infected-tissues with an isotonic sucrose solution to a new host which had previously fasted for 24 hours.

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