

The Effects of —10°C. and —16°C. on the Viability and Infectivity of *Trichinella spiralis* Larvae

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The utilization of low temperatures to destroy encysted larvae of *Trichinella spiralis* has been practiced for many years. It was felt that the work of previous workers (Augustine, D. L., 1933; Blair, J. B., and O. W. Lang, 1934; Gould, S. E., 1945; Nolf, L. O., and J. M. Edney, 1935; Ransom, B. H., 1916) lacked precision in that after treatment, viability of the experimental organisms was not checked by testing the infectivity of the larvae, or if checked, the work was not done in a comprehensive manner.

The primary purpose of the present experiments was to test the resistance of *T. spiralis* larvae (encysted in rat muscle) to low temperature effects and to determine whether they are, if viable, capable of producing an infection, by means of comprehensive feeding experiments involving young, susceptible, laboratory-reared albino rats.

Experiment 1 was designed to verify the period required for larvae of *T. spiralis* to develop to sexual maturity and produce infective larvae in the diaphragm of albino rats. Each of twelve, fifty-day-old rats were fed 1100 larvae. Two of these were killed on each of the following days after infection: 14, 16, 17, 18, 21, 28. The diaphragms were removed, digested, and observations made for the presence of larvae (Table I).

Experiment 2 was planned in an attempt to demonstrate the resistance of different age larvae to —16°C. Eighteen fifty-day-old rats were fed 1100 larvae each. Two of these were autopsied on each of the following days after infection: 18, 21, 28, 35, 42, 49, 63, 94, 100. The diaphragms of each of these was exposed to —16°C. for three minutes. One hundred-fifty of the larvae recovered from each diaphragm were fed to individual rats (50-70 days old). These test rats were sacrificed 30 days after infection and the larvae digested and counted (Table II).

Experiment 3 was conducted to determine the margin of safety provided by an exposure of —16°C. for three minutes as used in experiment 2. Four rats were heavily infected with *T. spiralis* and autopsied when the infections were one hundred days old.* The diaphragms of two of these were exposed to —16°C. for three minutes and those from the other two rats were exposed to the same temperature for one minute. Then three hundred of the larvae digested from each diaphragm were fed to individual fifty-day-old rats. Thirty days after infection these animals were sacrificed and the diaphragms were examined for the presence of encysted larvae (Table III).

Experiment 4 was designed to demonstrate the resistance of well established (70-day) infections of larvae of *T. spiralis* to —10°C. Sixteen fifty-day old rats were heavily infected with *T. spiralis* and sacrificed when the infections were seventy days old. These experimental animals

* The age of infections cited herein was reckoned from the time of ingestion of infective larvae.

TABLE I. Period required for larvae of *T. spiralis* to develop to sexual maturity and produce ineffective larvae in the diaphragm of albino rats.

Rat No.	Days After Ingestion of Infective Larvae	Larvae Present in Diaphragm
1	14	0
2		0
3	16	0
4		0
5	17	0
6		0
7	18	7
8		10
9	21	26
10		34
11	28	720
12		112

were divided into groups of four each. The diaphragms of one group were subjected to -10°C . for one minute, those of the second group were exposed to this temperature for three minutes, the diaphragms of the third group were exposed to -10°C . for five minutes; the fourth group for ten minutes. Two hundred-forty of the larvae recovered from each diaphragm were then fed to each of sixteen fifty-day old rats. Eight fifty-day old rats were fed two hundred-forty untreated larvae as controls. These were autopsied when the infections were thirty days old and the recovered larvae were counted (Table IV).

Results and Discussion

The twelve albino rats, each of which was infected with 1100 larvae of *T. spiralis* in experiment 1, first showed the presence of larvae in the diaphragm on the eighteenth day after infection. This agrees very closely with the findings of Nolf and Edney (1935) who established infections after feeding trichinous muscle from rats which had been infected seventeen days previously.

Apparently, the larvae of *T. spiralis* begin to attain infectivity by the time they enter the diaphragm in experimental infections. This is

TABLE II. Resistance of different-age larvae after exposure to -16°C . for three minutes as tested by feeding 150 of treated larvae to 50-70 day old rats and recovering second generation larvae from the diaphragms in 30 days.

Rat No.	Age of Larvae in Days After Infection	Test Rat No.		No. of Larvae Recovered	
		Expt.	Control	Expt.	Control
1	18	19	37	0	7
2		20	38	0	10
3	21	21	39	0	26
4		22	40	0	34
5	28	23	41	0	720
6		24	42	0	112
7	35	25	43	0	101
8		26	44	0	284
9	42	27	45	0	246
10		28	46	0	68
11	49	29	47	0	204
12		30	48	0	138
13	63	31	49	0	78
14		32	50	0	66
15	94	33	51	0	93
16		34	52	0	116
17	100	35	53	0	82
18		36	54	0	109

supported by the fact that one hundred-fifty larvae obtained from this muscle of rats which had been infected eighteen days previously, produced light infections when fed to test animals. There is a progressive increase in infectivity from the eighteenth to the twenty-eighth day after infection as shown on Table I.

A three-minute exposure at -16°C . was selected as the first in a series of experiments to demonstrate the resistance of *T. spiralis* larvae of different ages to low temperature effect. This was considered a reliable starting point on the basis of experiments by previous investigators.

It is significant that one hundred per cent of the larvae in experiment 2, from infections ranging in age from eighteen to one hundred days, were killed at -16°C . during an exposure of three minutes (Table II). The discrepancy between low-temperature requirements for production of one hundred per cent lethality in the rat host as demonstrated by the work of Blair and Lang (1934) and the present investigator, is maximum. There are several possible explanations which may be applicable here. Firstly, Blair and Lang interpreted viability by observation of motility which is proven fallacious by the present study. In each case in experiment 2, the larvae were motile when fed to the test animals but, as shown in Table 2, none produced an infection. Secondly, fast freezing may not be as detrimental as fast thawing which was accomplished in the present study through the use of physiological saline at room temperature. Blair and Lang thawed their material in the air at room temperature. Thirdly, the drop in temperature may have been faster in the present study than in the work of Blair and Lang.

After obtaining one hundred per cent lethality at -16°C . during a three-minute exposure, the author decided to determine the margin of safety provided by this procedure. Therefore, trichinous diaphragms harboring one-hundred day old infections were exposed to -16°C . for one- and three-minute periods. The production of infections by trichinous diaphragms exposed for one minute demonstrated the margin of safety to be narrow (Table IV).

TABLE III. Effects of exposure to -16°C . (for 1 and 3 minutes) on 100-day old *T. spiralis* larvae as evidenced by feeding 300 of the experimental larvae to individual 50-day old test rats, and presence of larvae in diaphragms after 30 days.

Rat No.	Exposure Time in Minutes	Test Rats No.	Recovered Larvae from Diaphragms
1	3	5	0
2		6	0
3	1	7	17
4		8	21

TABLE IV. Resistance of larvae from 70-day old infections of *T. spiralis* after exposure to -10°C . for varying periods of time, as revealed by larvae recovered from the diaphragms of 50-day old test rats 30 days after each was infected with 240 treated larvae.

Exposure in Minutes	Rats		Larvae Recovered	
	Expt.	Control	Expt.	Control
10	1	17	0	121
	2		0	
	3	18	0	109
	4		0	
5	5	19	0	121
	6		24	
	7	20	14	109
	8		5	
3	9	21	15	76
	10		9	
	11	22	81	56
	12		33	
1	13	23	54	69
	14		68	
	15	24	83	71
	16		175	

Having discovered marked lethal effects to larvae of *T. spiralis* during an exposure of -16°C . for three minutes, experiment 4 was performed to test the resistance of well-established (70-day) infections of these larvae to -10°C . Table IV shows that viability at this temperature as revealed by feeding experiments varied, inversely as the period of exposure. Ten minutes at this temperature produced one hundred per cent lethality.

Summary

1. Infective larvae of *Trichinella spiralis* first appear in the diaphragm of the rat host on the eighteenth day after ingestion of trichinous material.

2. *T. spiralis* larvae begin to attain infectivity by the time they are present in the diaphragm in experimental infections. Infectivity increases progressively from the eighteenth to the twenty-eighth day after infection.

3. A rapidly attained temperature of -16°C . for three minutes in conjunction with quick thawing is one hundred per cent lethal to infections of *T. spiralis* larvae ranging in age from eighteen to one hundred days. The margin of safety of an exposure to -16°C . for three minutes is narrow since one-minute exposures at the same temperature are not one hundred per cent lethal to trichina larvae.

4. Feeding experiments suggest that motility is fallacious as a criterion of infectivity.

5. Viability of *T. spiralis* larvae exposed to -10°C . for one, three, five, and ten minutes varies inversely as the period of exposure. A ten-minute exposure at this temperature kills *Trichinella spiralis* larvae.

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