# EFFECTS OF HEAT ON TRICHINÆ

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## INTRODUCTION

It is a well-known fact that the larvæ of *Trichinella spiralis*, which are of rather common occurrence in pork, may be killed by thorough cooking and the meat thereby rendered safe for food so far as concerns the danger of trichinosis. As to the actual temperature required to kill the parasites, however, various writers give very different figures, so that the question of the thermal death point has been rather uncertain.

The thermal death point of trichinæ is a matter of great practical importance in connection with the control of cooking processes employed by meat-packing establishments in the preparation of cooked products containing pork. The simple rule of cooking pork until it is well done, which can be applied satisfactorily by a careful cook in the household kitchen, is not suited to conditions in meat-packing establishments. Instead of such a rule a more exact statement of requirements is desirable. In fact, the Bureau of Animal Industry, which is charged with the enforcement of the federal meat-inspection law, requires that pork or products containing pork cooked in establishments operating under Federal inspection shall be heated sufficiently to insure a temperature throughout all portions of the meat that will destroy the vitality of any trichinæ which may be present, specifically a temperature of 137° F. (58° + C.). This temperature is several degrees higher than the temperature that has been accepted by the bureau as representing the thermal death point of encysted trichinæ, but the difference between the two represents no more than a reasonable allowance as a margin of safety.

Before a decision could be reached as to the degree of heat required to destroy the vitality of encysted trichinæ, it was found necessary to supplement the investigations on this question which are recorded in the literature with further experimental work; and it is the purpose of this paper to set forth the results obtained. This work was begun by the senior writer in 1913, continued in 1914 and 1915, and in the latter part of 1915 taken up by the junior writer.

## REVIEW OF LITERATURE

Haubner, Küchenmeister, and Leisering (*5*) [1] state that trichinæ are killed by prolonged salting, followed by 24 hours of smoking, but do not give data as to the temperature of smoking.

[1] Reference is made by number (italic) to "Literature cited," pp. 220–221.

Fiedler (*1, p. 26-29*) found that if small particles of trichinous meat were heated to 35° R. (43.75° C.) in water the heating had no other effect than to render the parasites more active when viewed at the same temperature under the microscope. Similar results were obtained by heating to a temperature of 40° R. (50° C.). The trichinæ in finely chopped meat held at a temperature of 50° R. (62.5° C.) for 15 minutes and then cooled were found to show movement when gently warmed, but reexamination of the meat 24 hours later failed to show any trichinæ that would move when warmed. This experiment was frequently repeated with similar results, and similar results were obtained with a temperature of 52° R. (65° C.). Temperatures of 58° R. (72.5° C.) and upward, allowed to act for a period of 10 minutes in all cases, affected the parasites so that no movement occurred afterward when gentle heat was applied. Three rabbits and a cat were fed trichinous meat after it had been heated 10 minutes at a temperature of 50° R. (62.5° C.), and none became infected. Trichinous meat heated 10 minutes at a temperature of 40° to 42° R. (50° to 52.5° C.) infected a rabbit. In another experiment meat heated at 40° R. (50° C.) for 10 minutes failed to infect a young cat. Trichinous meat heated at 60° R. (75° C.) for 10 minutes failed to infect two rabbits.

In another paper Fiedler (*2, p. 467-468*) reported an experiment in which he fed two rabbits with minced trichinous meat that had been heated in water for 10 minutes at a temperature of 50° R. (62.5° to 65° C.). No infection resulted. He also reported an experiment in which two rabbits were fed with trichinous meat that had been heated in water for 10 minutes at a temperature of 45° to 46° R. (56.25° to 57.5° C.). No infection resulted.

Haubner (*4*) states that the smoking of pork at a temperature which reaches and exceeds 52° R. (65° C.) kills the trichinæ or brings about their early death.

Rodet (*12*) states that trichinæ do not die at a temperature of 55° to 60° C. He also asserts that they survive even a temperature of 70° to 80° C. and succumb with certainty only to a temperature of 100° C. In support of his views Rodet presents very imperfect experimental evidence. He states that he placed pieces of trichinous muscle in water at a temperature of 70° to 80° C. and allowed them to remain there for some time. Upon being taken out of the water the trichinæ in the meat were still lively. When plunged into water at 100° C. they were killed and became completely uncoiled.

Fjord and Krabbe (*3*) concluded that encysted trichinæ die at 52.5° C. after a 30 minutes' exposure. At 54° C. they survived 10 minutes and at 55° to 56° C. they died in 5 minutes. Their method of procedure consisted in cutting up trichinous meat and heating it in a vessel containing warm water while agitating the contents with a thermometer. To

determine the effects of the heating upon the vitality of the parasites they fed the meat to rabbits, which were examined for trichinæ 15 to 30 days after feeding.

Perroncito (7) records observations on the behavior of the larvæ under the influence of high temperatures and draws the conclusion that a temperature of 48° to 50° C. is sufficient to kill the parasites. He placed decapsuled larvæ as well as encysted larvæ in salt solution and examined them on a warm stage. He observed that as the temperature increased the larvæ became more active, but that at 45° C. their activities ceased. If the temperature was lowered they resumed their activities. If the temperature was raised to 48° or 50° C. they became completely inactive and remained so even when the temperature was lowered.

Vallin (*13*) records a series of experiments on the effects of heat on trichinæ. He heated small pieces of trichinous meat in tubes containing water, placed the tubes on a sand bath, and read the temperatures on a thermometer with which each tube was provided. He found that a 20-minutes' exposure to a temperature of 60° C. resulted in a complete destruction of the vitality of the larvæ. He fed the heated meat to two rabbits and four guinea pigs and failed to infect them. Vallin states that temperatures below 60° C. are uncertain in their effects, since after heating meat to 56° C. he succeeded in infecting with it one guinea pig, although two rabbits to which the meat was fed escaped infection. He tried temperatures lower than 56° C. and found them ineffective.

Leuckart (*6*) states that *Trichinella spiralis* does not perish until it is acted on by a temperature ranging between 62° and 69° C.

Piana (*8*) concluded as a result of certain experiments that a temperature of 56° C. is fatal to the larvæ of *Trichinella spiralis*.

Ransom (*10, p. 159*) states:

> With reference to the effects of high temperatures upon the vitality of trichinæ, various statements are found in the literature which seem to have for the most part rather imperfect experimental evidence as a basis. From a rather small series of experiments conducted within the last two years, I have found that encysted trichinæ regularly die when exposed for a short time to a temperature somewhere between 53° and 55° C.

The earlier of these experiments supplied the data upon which was based the following statement (*9*): "The results already obtained in the investigations . . . show that the parasites die after a brief exposure to a temperature between 53° and 55° C."

Winn (*14*) records a series of experiments in which trichinous meat was heated to certain temperatures, maintained at those temperatures for 15 minutes, and then fed to experimental animals. The effect of the heat was judged by the degree of infection as compared with that of animals fed on similar quantities of meat which were unheated. Winn found that temperatures below 53° C. produce no apparent effect upon

the vitality of the worms. At 53° C. he found the vitality of the worms slightly reduced, but the results were variable. At 54° C. there was a further reduction in vitality, but meat which was heated to 55° C. and maintained at that temperature for 15 minutes was not capable of producing an infection.

## EXPERIMENTAL WORK

Experiments by the present writers on the effects of heat on the larvæ of *Trichinella spiralis* have been made with meat containing encysted larvæ as well as with larvæ freed from their capsules by artificial digestion. In the former case there is more or less difficulty in obtaining accurate data, since the temperature in the interior of the meat does not necessarily correspond to the temperature of the medium in which it is heated. This difficulty may be overcome, however, if small pieces of muscle tissue are used and if the temperature is raised gradually. In experiments on larvæ freed from their cysts by artificial digestion more accurate determinations can be made, since the temperature of the medium is an excellent index to the temperature of the parasites themselves. From a comparison of the results obtained by the two methods definite conclusions regarding the thermal death point of the larvæ may be drawn.

## OBSERVATIONS ON THE SURVIVAL OF DECAPSULED LARVÆ IN VARIOUS MEDIA

In connection with experiments on the effects of heat upon decapsuled larvæ, the question of their survival in various media following artificial digestion is important, since such experiments are complicated by the factor of abnormal environment, and results obtained might not correspond with those obtained in experiments in which the parasites are subjected to heat while still inclosed in their capsules in pieces of meat. Encysted trichinæ may be kept alive for many months and may still be viable in meat that has become badly decomposed. Although decapsuled larvæ are unlikely to survive as long as encysted larvæ, they can be kept alive for considerable periods of time. In a paper by the senior writer (Ransom, *11*), it has been shown that decapsuled larvæ may retain their normal activity and appearance when kept in tap water or 0.6 per cent salt solution at a temperature of about 20° C. for a period of from 10 days to two weeks or more, and that they have been kept alive and very active for as long as 11 days in 2 per cent salt solution. On the other hand, at a temperature of 38° decapsuled larvæ kept in tap water became inactive within a few hours, whereas when kept in 0.6 per cent salt solution at the same temperature for the same length of time they suffered no apparent injury.

Further observations have been made by the junior writer which show quite clearly that the longevity of the larvæ after artificial digestion depends upon both the medium in which they are kept and the temperature

to which they are subjected. Pure water as compared to physiological salt solutions was found to be distinctly injurious, the injurious action varying directly with the temperature. Larvæ kept in distilled water at a temperature of 39°–40° C. were all dead at the end of 22 hours, while in 0.7 per cent solution of sodium chlorid or in Ringer's solution they lived longer, although they all died within 48 hours. In distilled water at a temperature of 32°–33° decapsuled larvæ were nearly all uncoiled at the end of 48 hours, while in 0.7 per cent sodium-chlorid solution or in Ringer's solution some were still alive at the end of 5 days. Similar differences were observed in the case of lower temperatures. In distilled water at 25°–26° larvæ remained alive for 4 days; in physiological salt solutions at 25°–27° some were still alive at the end of 13 days; in distilled water kept at a temperature of about 8° only a few larvæ were still alive at the end of 12 days; while in physiological salt solutions at the same temperature some larvæ were still alive at the end of 50 days.

From these observations and our general knowledge of the phenomena of osmosis it would appear that the loss of salts from the tissues of the worms into the water and the penetration of the water into the tissues of the worms are important factors in bringing about the death of the worms when kept in hypotonic media, such as distilled water. This belief is borne out also by the fact, noted in a former paper (*11, p. 849*) and repeatedly observed since that paper was written, that larvæ kept in a hypotonic solution until they have begun to show distinct evidence of its effects, such as loosening of their coils and paling of their protoplasm, if transferred to a physiological salt solution before the injurious action of the hypotonic medium has gone too far, will usually resume a normal state of contraction and a normal or almost normal brown color. Another indication that the death of decapsuled larvæ kept in hypotonic solutions may be dependent upon osmotic processes is that they die more quickly at high than at low temperatures, which is in harmony with the fact that osmosis is hastened by raising the temperature.

Another factor or factors, however, enter into the matter, inasmuch as in isotonic solutions as well as in hypotonic solutions the larvæ do not survive so long at high temperatures as at low temperatures. It may be supposed that at the higher temperatures death of the larvæ kept in isotonic and comparatively inert solutions is brought about by exhaustion resulting from the greater activity of the worms and consequently more rapid oxidation of their tissues than at lower temperatures. Such an explanation is complicated by the fact that larval trichinæ encysted in the muscles of a living animal may live for many years, although constantly subjected to a temperature at which they live only two or three days when removed from their cysts and kept in salt solutions. Possibly in the living animal they are kept in a relatively inactive condition through the operation of factors no longer present when they are removed

from their normal environment, and it is possible also that they may be able to replace waste through the absorption of nutritive materials from their host.

A natural corollary to experiments on the effects of hypotonic solutions are experiments on the effects of hypertonic solutions. A typical example of such an experiment is one in which decapsuled larvæ were kept for 22 hours in a molar solution of dextrose. At the end of this time they were found to be partially uncoiled; their protoplasm was dull in appearance; the cuticle was wrinkled, particularly in the posterior portion of the body; the body wall was wrinkled; and the cells of the esophagus were indistinct. After having been transferred to and kept in 0.7 per cent salt solution overnight, they were found to be tightly coiled and normal in appearance. Similar results were obtained in a repetition of this experiment.

So far as concerns the purposes of the present paper, the foregoing observations are of interest because they show that trichinæ freed from their cysts by artificial digestion may be kept alive for a long time in physiological salt solutions, in water, and in certain hypertonic solutions, and that, although within a temperature range the upper limit of which does not exceed 40° C. their longevity decreases as the temperature at which they are kept is raised, they do not in any case die quickly.

## EXPERIMENTS WITH DECAPSULED LARVÆ

Inasmuch as trichina larvæ that have been freed from their cysts by digestion of finely chopped trichinous meat in artificial gastric juice[1] at a temperature of 38° to 40° C. for a period of about 20 hours can be kept alive for long periods of time, they can be conveniently used in experiments on the effects of heat. In a medium such as a 0.6 per cent or 0.7 per cent solution of sodium chlorid, but also in plain water if not kept too long, they display more or less activity even at ordinary room temperatures but commonly assume a posture in which they are tightly coiled spirally; and their movements are often limited to a tightening or loosening of the coil. Their protoplasm, when unaffected by heat or other injurious agents, exhibits a certain brilliancy in appearance; and pigment in the cells of the alimentary tract, especially of the esophagus, gives them a distinct brownish color. After a little experience, departures from the normal both as to their behavior and appearance of their protoplasm can easily be detected by microscopic examination. As a rule, in experiments in heating decapsuled larvæ, the larvæ were placed in a beaker or test tube containing sometimes water but usually a phys-

[1] The following fluid has yielded satisfactory results:

| | |
|---|---|
| Scale pepsin (U. S. P.) | 2.5 gm. |
| Sodium chlorid | 2 gm. |
| Hydrochloric acid (sp. g. 1.19) | 10 cc. |
| Water | 1,000 cc. |

iological salt solution or Ringer's fluid; and this was heated to the desired temperature on a water bath over an open flame, or in an incubator. After being cooled, individuals were removed with a pipette to hollow ground slides, or in some cases transferred to a Petri dish or shallow stender dish and allowed to cool. They were then examined directly on a warm stage, either on slides or in the dishes, in order to determine the results of the experiment.

### BEHAVIOR OF DECAPSULED LARVÆ WHEN HEATED

When trichina larvæ are heated on a warm stage their reactions may be directly observed with the microscope. As the temperature rises they begin to uncoil and become very active, their activity gradually increasing. When the temperature has reached the neighborhood of 50° C. spasmodic contractions are commonly observed, and the larvæ twist themselves into various shapes. With a further rise of temperature they grow sluggish and may become either uncoiled and inactive or else tightly coiled and quiescent. After passing into this sluggish condition they may again become lively if the temperature is lowered, but if subjected to a sufficiently high temperature for a sufficient length of time they do not recover when removed to a cool place.

Decapsuled trichinæ killed by heat usually become uncoiled and assume a characteristic shape resembling the figure 6. If allowed to stand for some time the protoplasm becomes dull, certain granulations appear, and often the cell partitions in the gonads can no longer be distinguished. Larvæ in this condition are readily recognizable as dead. Sometimes, however, larvæ that have been subjected to heat may remain loosely coiled and the protoplasm may not undergo any conspicuous changes. From experience it has been learned that larvæ in this condition are usually dead. A generally satisfactory test of life is heat stimulation; if still viable the larvæ will usually uncoil and move. Even individuals with a minimum amount of vitality will move the anterior or posterior end very sluggishly. However, the most reliable test of life, or at least of their viability from a practical standpoint, is feeding them to experimental animals and thus determining their ability to reproduce; and this has been done in some instances but not so regularly as in experiments on encysted trichinæ.

### DETAILS OF EXPERIMENTS

Some experiments on the effects of heat on decapsuled trichinæ were made by the senior writer in 1913, 1914, and 1915, after which the work was taken up by the junior writer and continued along the same general lines.

EXPERIMENT 1 (April 5 and 7, 1913).—A decapsuled larva was sealed under a cover glass in salt solution on a slide and heated to 54° C. on a

warm stage. The temperature was held at 54° for a few moments. The worm was inactive at this temperature but resumed its movements when the slide was cooled. The same worm was reheated to 55° and became entirely motionless at this temperature. The temperature was raised to 55.5° and the slide then cooled. The worm became active again on cooling.

Another decapsuled larva was heated in the same manner. It became sluggish in its movements and coiled up at a temperature of 48° C. The temperature was raised slowly to 56°, and the slide was allowed to cool as soon as this temperature was attained. The worm resumed its active movements when cooled. In order to check the correctness of the temperature indicated by the thermometer in this experiment, some crystals of diphenylamin having a melting point of 54° were placed on a slide under a cover glass and heated on the stage. They melted when the thermometer registered 54°. A second trial gave the same result.

On April 7, a decapsuled larva was heated as described above. The temperature was raised slowly to 56° C. and then held for five minutes at 56° to 56.5°. When cooled the worm did not resume its movements, its internal structure showed slight disorganization, and it was undoubtedly dead.

EXPERIMENT 2 (March 28, 1914).—Decapsuled trichinæ, isolated by artificial digestion from a mixture of meat from three trichinous rats, were heated in a beaker of constantly stirred water over a hot water bath to a maximum of 53.6° C., 10 minutes being required for the temperature to rise to this point from 30°. The temperature dropped to 46.2° in another 10 minutes, after which 233 larvæ were examined at room temperature. All were inactive. Unheated larvæ from this lot when examined at room temperature were active. Another lot of larvæ from the same source was heated in the same manner, the temperature rising from 20° to 51° in 21 minutes, and then dropping in 6 minutes to 45.8°. One hundred and thirty-nine larvæ were then examined at room temperature, and 65 of them were found to be inactive. Of the 74 active larvæ, all but 2 were sluggish. A third lot of larvæ from the same source was heated in the same manner from 24° to 50° in 12 minutes, and then cooled to 46° in 6 minutes. Out of 159 examined, 18 were inactive. Some of the 141 active larvæ were sluggish.

EXPERIMENT 3 (May 16, 1914).—Decapsuled larvæ, isolated by artificial digestion from a mixture of meat from two trichinous rats, were heated in a beaker of constantly stirred water over a water bath. The temperature was raised from 23° to 48.4° C. in 8 minutes and held at 48.4° 1 minute. The beaker was then allowed to cool. One hundred and ten larvæ were examined on a warm stage. Thirty-five were inactive and 75 active, mostly very lively. Another lot of larvæ from the same source was heated in the same manner from 22° to 51° in 10 minutes.

Examination of 213 larvæ on a warm stage showed 179 inactive and 34 active, most of them very lively. Another lot was heated from 30° to 51.9° in 10 minutes. Ninety-nine were examined, and of these 93 were inactive and 6 active. Another lot was heated from 30° to 53° in 4 minutes. One hundred and eighteen were examined, and of these 72 were inactive and 46 active, sluggish. Another lot was heated from 22° to 53° in 12 minutes. One hundred and forty-seven were examined, and of these 109 were inactive and 38 active, sluggish. As a control upon the results of this experiment 158 unheated larvæ from the same source as those subjected to heat were examined on a warm stage. Of these 22 were inactive and 136 active.

EXPERIMENT 4 (November 17, 1914).—Decapsuled larvæ, isolated by artificial digestion from the meat of a trichinous hog, were heated in a beaker of water over a hot water bath for a period of 10 minutes, during which time the temperature gradually increased from 23° to 53.4° C. The beaker was then cooled. Seventeen of the larvæ were examined on a warm stage and one was observed to move slightly. Fifteen minutes later the larvæ remaining in the beaker were reheated to a temperature of 53.6° C., seven minutes being required to raise the temperature to this point from 38°. Twenty-four larvæ were examined after this reheating; one exhibited definite movements on a warm stage. The others were more or less tightly coiled and presumably still alive. Thirteen minutes later the larvæ remaining in the beaker were heated a third time, the temperature being raised rapidly (in 3 minutes) from 43° to 55°. Thirty-nine larvæ were examined; all were motionless and failed to react to heat, evidently dead.

EXPERIMENT 5 (November 17, 1914).—Decapsuled larvæ from the same source as those used in Experiment 4 were heated in the same manner from 16° to 54° C., 7½ minutes being required for raising the temperature. Twenty-three larvæ were examined after heating and all were found to remain inactive on a warm stage. The remainder of the larvæ in the beaker were left on the laboratory table until the following day when 42 of them were examined on a warm stage heated to 45°. Most of these were inactive but more or less tightly coiled. Thirty-five others were placed on a warm stage heated to 61°. Six of these exhibited convulsive movements before they succumbed to the heat, the others showing no response to stimulation.

EXPERIMENT 6 (November 17, 1914).—Decapsuled trichinæ, isolated by artificial digestion from a mixture of meat from six trichinous hogs, were heated in a beaker of water over a hot water bath to a temperature of 53.4° C. Some of them showed signs of life when examined on a warm stage. The beaker was reheated to 55°. Fifty larvæ were then examined on a warm stage and all were found to be dead.

EXPERIMENT 7 (December 19, 1914).—Decapsuled larvæ, isolated by artificial digestion from meat of a trichinous hog, were heated in 0.6 per cent salt solution in a corked bottle over a water bath. The temperature, determined by a thermometer inserted through the cork, rose from 24.4° to 56.7° C. in 44 minutes and remained at this maximum for 30 seconds, after which the bottle was allowed to cool, the temperature dropping to 34.4° in 38 minutes. Three hundred and sixty-five of the larvæ were then examined on a warm stage and all were found to be inactive. As a control on the results of this experiment 22 unheated larvæ from the same source were examined on a warm stage; 4 were inactive, 18 active.

EXPERIMENT 8 (April 6, 1915).—Decapsuled trichinæ, isolated by artificial digestion from a mixture of meat from six hogs, were kept 7 days in 0.6 per cent salt solution at ordinary room temperature. Some were then heated in a beaker of the salt solution, constantly stirred, over a water bath. The temperature rose from 20° to 54° C. in 7 minutes, and remained at this maximum for 30 seconds, after which the beaker was allowed to cool. Examination of some of the larvæ from the beaker showed that most of them were more or less uncoiled, but some were tightly coiled and practically normal in appearance. The beaker was kept until the following day at ordinary room temperature and the contents again examined. The great majority of the worms were still alive, but most of them were not tightly coiled.

Another lot of larvæ from the same source was heated in a similar manner but more slowly, the temperature rising from 23° to 54.8° C. in 56 minutes, remaining at 54.8° for 1 minute, after which the beaker was allowed to cool. Four hundred and seventy larvæ were examined; all were uncoiled, and their protoplasm was rather dull in appearance. The beaker was kept at room temperature until the following day, when examination of 200 larvæ showed that all were dead.

Subsequent experiments on the effects of heat on decapsuled larvæ were performed by the junior writer.

EXPERIMENT 9.—Decapsuled trichinæ in a physiological salt solution were placed in a test tube and a thermometer immersed in the solution. The test tube was placed in a beaker of water, which was heated rapidly until the thermometer registered 55° C. This temperature was attained in four minutes. The contents of the test tube were immediately transferred to a stender dish and allowed to cool. The larvæ were then examined. Nearly all were unaffected. A few days later this experiment was repeated, increasing the time of heating to about eight minutes. Similar results were obtained.

The results of other experiments with various lots of decapsuled larvæ are shown in the following table:

TABLE I.—*Effect of various temperatures on decapsuled larvæ*

| Maximum temperature. | Time required to reach maximum temperature. | Results. |
|---|---|---|
| °C. | | |
| 53 | Not recorded | Some alive. |
| 53 | 50 minutes | Nearly all alive. |
| 54 | Not recorded | Some alive. |
| 54 | ....do | Do. |
| 54 | 42 minutes | All dead. |
| 54.6 | 54 minutes | Do. |
| 54.8 | Not recorded | A few showing sluggish movements. |
| 55 | .....do | None active. |
| 55 | .....do | Do. |
| 55 | 77 minutes | All dead. |
| 55 | 60 minutes | All expanded. |
| 55 | 65 minutes | Do. |
| 55 | 37 minutes | All dead. |
| 56 | Not recorded | Do. |
| 56 | 52 minutes | Do. |
| 56 | 83 minutes | Do. |

EXPERIMENT 10.—A 0.6 per cent salt solution was heated to 56° C. At this point some decapsuled larvæ were spurted into the solution from a capillary pipette. The temperature dropped from 56° to 55° in 75 seconds, and the contents of the vessel were then emptied into a shallow dish and examined. Of 25 larvæ, 14 were uncoiled and 11 tightly coiled. The same experiment was repeated. Of 21 larvæ, only 3 were completely uncoiled. In another test the larvæ were spurted into the solution at 55° after which the temperature was allowed to drop to 54°, which required 85 seconds. On examination following transfer to a shallow dish, only 3 out of 18 larvæ were found to be completely uncoiled.

In order to control the results of direct examination of decapsuled larvæ after heating, the junior writer in two instances fed some of the larvæ to rats. Thus larvæ heated rapidly to 55° C. in Experiment 9 were fed to two rats, which when killed at the end of a month were found to be moderately infected, a result in agreement with the results of direct examination of the larvæ. In another case—one of the experiments summarized in Table I—larvæ heated gradually for 60 minutes to 55° were fed to two rats, which were found free from trichinæ a month later. Another rat fed unheated decapsuled larvæ from the same source became infected.

From the foregoing experiments it is evident that decapsuled trichina larvæ are killed by a temperature of 55° C., provided this temperature is gradually attained. Many may be killed by lower temperatures, but the results of heating to temperatures lower than 55° are uncertain. It is also apparent that a momentary exposure to a temperature of 55° is not sufficient to destroy the vitality of decapsuled larvæ, as is shown by the results of Experiments 1, 9, and 10.

## EXPERIMENTS WITH ENCYSTED LARVÆ

The experiments on decapsuled larvæ were supplemented by experiments on encysted larvæ in their natural location in pieces of infested muscle, the earlier of these experiments being made by the senior writer, the later, as noted, by the junior writer.

EXPERIMENT 11 (March 31, 1913).—Small pieces of meat from a trichinous rat were placed in a beaker of water (about 500 cc.) in a constant-temperature oven. The temperature of the water increased from an initial temperature of 18.4° to 48.4° C. in 1 hour and 10 minutes, at which time a piece of the meat was removed. Ten minutes later, when another piece was removed, the temperature had reached 51°. Eleven minutes after this at a temperature of 52.8° another piece was removed. After another period of 15 minutes, when the temperature had reached 55°, another piece was removed. Thirty-seven minutes later, when the thermometer registered 59.8°, another piece of meat was removed. A few larvæ were isolated by dissection from these various pieces of meat and examined under the microscope. The larvæ from the pieces heated to 48.4° and 51° were alive and active. One out of four larvæ from the piece heated to 52.8° showed slight movements; the others were inactive. Those from the pieces heated to 55° and 59.8° were inactive when examined. The results of direct examination of the larvæ were checked by feeding the various pieces of meat to guinea pigs. The guinea pigs fed with the meat which had been heated to 48.4° and 51° became heavily infected; those fed the pieces heated to 52.8°, 55°, and 59.8° remained free from trichinæ.

EXPERIMENT 12 (April 1, 1913).—Several small pieces of rat muscle were placed in a vessel containing 500 cc. of water and heated in an oven from an initial temperature of 16° to a temperature that reached 55° C. at the end of two hours. Pieces of meat were removed at temperatures of 51.2°, 52.2°, 53°, and 55°. A few larvæ from each piece of meat thus removed were isolated and examined directly on a warm stage. Samples from these pieces of meat were also fed to guinea pigs, which were killed about a month after feeding. The direct examination of the larvæ on a warm stage showed that, with the exception of those from the meat heated to 55°, the majority were alive and responded to thermal stimulation. Those heated to 55° were loosely coiled and did not become active on the warm stage.

The results of the feeding experiments were as follows: The guinea pig that was fed meat heated to 51.2° C. was killed seven days after feeding because it became sick. The muscles were negative, but one pregnant female trichina was found in the intestine. The guinea pig that was fed meat heated to 52.2° was killed about five weeks after feeding, and only one encysted larva was found in the diaphragm. No parasites were found in the intercostal muscles. The guinea pig that was fed meat

heated to 53° was killed five weeks after feeding and was free from parasites. The meat heated to 55° also failed to infect two guinea pigs.

EXPERIMENT 13 (April 1, 1913).—Small pieces of meat from a trichinous rat were heated as in the previous experiment; but an open flame was used instead of an oven and the temperature was allowed to go up very rapidly, the water in the beaker meanwhile being stirred constantly. Meat heated from 27.8° to 53° C. in 3½ minutes was fed to a guinea pig and resulted in a mild infection. Meat heated from 27.8° to 52° in 3 minutes and from 20° to 49.2° in 6 minutes when fed to guinea pigs produced heavy infections.

EXPERIMENT 14 (April 3 and 4, 1913).—A small piece of meat from a trichinous rat was heated in a beaker of water which was constantly stirred. The temperature rose from 17° to 53° C. in 13 minutes and remained between 53° and 53.6° for 2 minutes. One larva afterwards isolated by dissection was inactive except at the anterior end which moved slightly; another was active, though the appearance of its protoplasm was somewhat altered.

Another piece of meat was similarly heated from about 20° to 54° C. in about 10 minutes. Larvæ isolated by dissection were alive and active. Another piece was similarly heated from 28° to 53° in 11 minutes and remained in the water another minute, during which time the temperature rose to a maximum of 53.8°. Larvæ isolated by dissection were alive and active. Two pieces were heated from 28° to 55° in 13 minutes. One piece was held at a temperature of 55° for 1 minute, the other piece at the same temperature for 2 minutes. Trichinæ isolated by dissection from these pieces were inactive. Another piece of meat from the same rat was heated from 30° to 54° in 5 minutes and held at a temperature of 54° to 54.8° for 1 minute. Larvæ isolated by dissection were found to be inactive.

EXPERIMENT 15 (April 9, 1913).—Small pieces of meat from two trichinous rats were tied in a cloth around the bulb of a thermometer, which was immersed in a beaker of water and heated. The temperature was held at 54.6° to 54.8° C. for five minutes. Ten larvæ were afterwards isolated by dissection. All were inactive except one, which showed a very slight movement of its anterior end.

EXPERIMENT 16 (May 16 and 19, 1914).—Portions of the diaphragm of a trichinous rat were heated in a beaker of water stirred constantly over a water bath. Trichinæ were dissected out of the meat after heating and examined under the microscope at room temperature. A portion was heated from 24° to 54° C. in four minutes. Four larvæ examined; 1 inactive; 3 active, sluggish. Another portion was heated from 24° to 53° in 6 minutes. Ten larvæ examined; all active. Another portion was heated from 23° to 54° in 5 minutes. Twelve larvæ examined; 3 inactive; 9 active but very sluggish; appearance of protoplasm abnormal.

In the following tests portions of the diaphragm of another rat were heated. A portion was heated from 24° to 54° C. in 5½ minutes. Ten larvæ examined; 9 inactive; 1 active, very sluggish. A portion was heated from 24° to 52° in 4¾ minutes. Ten larvæ examined; all active, lively. A portion was heated from 24° to 58° in 3¼ minutes. Ten larvæ examined; all inactive. A portion was heated from 26° to 53° in 3½ minutes. Five larvæ examined; all active but not very lively. A portion was heated from 26° to 55° in 4 minutes. Twenty-three larvæ examined; 21 inactive; 2 active, very sluggish. A portion was heated from 24° to 52.6° in 9 minutes. Twelve larvæ examined; 2 inactive; 10 active, but very sluggish; appearance of protoplasm abnormal. A portion was heated from 23° to 52.9° in 2½ minutes. Eight larvæ examined; all lively. A portion was heated from 22° to 52° in 3¾ minutes. Twenty-four larvæ examined; all lively.

EXPERIMENT 17 (May 20, 1914).—Portions of the diaphragm of a third rat were heated as in Experiment 16, but more gradually. Examination was made as in Experiment 16. A portion was heated from 26° to 53° C. in 12½ minutes and cooled to 48.8° in 5 minutes. Sixteen larvæ examined; all active, but sluggish; appearance of protoplasm duller than normal. A portion was heated from 23.2° to 52° in 14 minutes and cooled to 46° in 7 minutes. Thirteen larvæ examined; all active, fairly lively but not as vigorous as unheated larvæ; no conspicuous change in appearance of protoplasm; larvæ not coiled as tightly as normal larvæ. A portion was heated from 23° to 55° in 16 minutes and cooled to 50° in 5 minutes. Fifteen larvæ examined; all inactive; protoplasm dull and dead in appearance. A portion was heated from 37° to 54° in 9 minutes and cooled to 49.4° in 6 minutes. Twenty-three larvæ examined; all active but very sluggish; protoplasm dull and dead in appearance. A portion was heated from 27° to 54° in 11¾ minutes and cooled to 49° in 5 minutes. Twenty-four larvæ examined; 16 inactive; 8 active but very sluggish; protoplasm dull and dead in appearance.

Experiments on encysted trichinæ were made by the junior writer as follows:

EXPERIMENT 18.—Small pieces of meat from a rat killed one month after infection with trichinæ were heated in a physiological salt solution to 52°, 53°, 54°, and 55° C., respectively, and then allowed to stand in a refrigerator for two days. The larvæ were then freed from their capsules by teasing out the meat, and examined directly. Those heated to 52° were still tightly coiled, although a number of loosely coiled larvæ were also seen. Most of the larvæ heated to 53° were uncoiled, but a few were coiled normally. Those heated to 54° and 55° were entirely uncoiled, dull in appearance, and failed to become active when warmed.

EXPERIMENT 19.—Larger pieces of meat from a trichinous hog were heated as in the experiment just described, kept in a refrigerator for two days, and then fed to mice. The post-mortem examinations yielded negative results in all cases.

The results obtained from the experiments in which pieces of trichinous meat were heated agree with the results of those in which the larvæ were first freed from their cysts by artificial digestion and then heated in water or physiological salt solution. The larvæ are killed if the meat is gradually heated to a temperature of 55° C., though some may escape if the temperature rises rapidly to 55° and soon falls again. They may survive a temperature of 54°; but meat which has been exposed to a temperature of about 53°, gradually attained, is likely to be non-infective.

It may be concluded that meat which has been heated so that the temperature throughout reaches 55° C. (131° F.) will be innocuous so far as concerns the possibility that persons eating such meat will become infected with trichinæ, inasmuch as under ordinary conditions of cooking the rise of temperature will be gradual enough to insure the destruction of the parasites if the temperature of the meat actually reaches 55° C. or higher. Under the regulations of the Bureau of Animal Industry the minimum temperature that must be attained throughout all portions of pieces of pork or products containing pork that are cooked in establishments operating under federal meat inspection has been fixed somewhat higher than 55° C., namely 137° F. (58.33° C.), which allows a margin of safety of several degrees above the temperature that has been shown by our investigations to be fatal to trichinæ.

## THE EFFECTS UPON TRICHINÆ OF CONTINUED EXPOSURE TO HEAT AT TEMPERATURES BELOW THE THERMAL DEATH POINT

It has been shown that trichina larvæ are killed by brief exposure to a temperature of 55° C., gradually attained; and since they will not afterwards resume their activity when thus heated, this temperature may be considered the thermal death point. The vitality of the larvæ may be destroyed also by exposure to lower temperatures, provided the application of heat is long enough continued. In the former case it may be assumed that death results from irreversible coagulations of the protoplasm, in the latter case either as the result of coagulation changes which become irreversible if the heat acts for a sufficient period, or as the result of exhaustion following excessive activity to which the larvæ are stimulated by heat. We may, therefore, distinguish three ranges of lethal temperatures: The highest, in which death comes quickly from rapid and irreversible coagulations of the protoplasm; an intermediate range, in which death results probably from somewhat similar coagulation changes, changes, however, from which the parasites may more or less completely recover if the temperature is lowered before death occurs; and the lowest range, in which death is apparently brought about by exhaustion from increased activity.

The following experiments to determine the effects of the continued exposure of decapsuled larvæ to temperatures below 53° C. were carried out by the junior writer. The larvæ in 0.7 per cent salt solution or in Ringer's solution were first heated to a given temperature and then placed in an incubator at the same temperature for a given period. When taken out of the incubator the larvæ were kept at room temperature at least an hour before they were examined.

EXPERIMENT 20.—In one test the larvæ were all dead after exposure for three hours to a temperature of 48° C., but generally an exposure to a temperature of 48° for less then four hours failed to destroy their vitality. In every case, however, after they were heated four hours at a temperature of 48° they were all uncoiled, having assumed the shape of the figure 6; and they failed to react to heat stimulation.

EXPERIMENT 21.—When exposed to a temperature of 49° C. nearly one-half the larvæ in one lot were still alive at the end of two hours. Another lot from a different host animal succumbed to a similar treatment, but in no case did a briefer exposure to 49° prove effective. When subjected to 49° for 3¼ hours all the larvæ became completely uncoiled, rigid, and insensitive to thermal stimuli.

EXPERIMENT 22.—At a temperature of 50° to 50.6° C., the vitality of the larvæ was completely destroyed after an exposure of 1 hour and 20 minutes. At a constant temperature of 50° an exposure of 1½ hours proved fatal.

EXPERIMENT 23.—An exposure of one hour to a temperature of 52° C. was sufficient to destroy the vitality of decapsuled larvæ.

From the foregoing experiments it is evident that decapsuled trichina larvæ die in a comparatively short time when exposed to temperatures in the neighborhood of 50° C. and that the time required for their destruction increases as the temperature is lowered. If the results of these experiments are considered in connection with the question of the length of time that decapsuled larvæ survive at temperatures ranging below 40°, already discussed in this article, it may be concluded that between limits at which the larvæ become altogether quiescent because of the effects of heat on the one hand and of cold on the other their longevity varies inversely with the temperature. It would, however, not be safe to conclude from the experiments just described that exposure of trichina larvæ to the temperatures given for the stated periods of time would be sufficient in all cases to destroy the vitality of the parasites. It is not improbable that in these experiments the larvæ had already become somewhat exhausted as a result of abnormal activity during the process of artificial digestion, and furthermore it is possible that different lots of trichinæ vary considerably with respect to their store of vitality. The following experiments by the senior writer show that the vitality of encysted trichinæ as well as that of decapsuled

trichinæ may be destroyed by continued heating at temperatures lower than that which kills on brief exposure. Like the experiments with the decapsuled larvæ, however, they are not sufficiently extensive to allow definite conclusions to be drawn as to the periods of time necessary to insure the destruction of trichinæ exposed to temperatures lower than the thermal death point.

EXPERIMENT 24 (April 7, 1913).—A small piece of the diaphragm of the same rat which supplied the meat used in Experiment 14 was tied in a cloth around the bulb of a thermometer, which was immersed in a beaker of water heated to about 50° C. and the entire apparatus placed in a constant-temperature oven. The temperature, as indicated by the thermometer, varied from 50.2° to 51.6° during the two hours of heating the meat. Larvæ isolated from the meat by dissection were dead.

EXPERIMENT 25 (April 9, 1913).—Two small pieces of meat from the same rat used in Experiments 14 and 24 were tied in cloths around the bulbs of two thermometers and heated in a beaker of water as in Experiment 24. During the experiment the temperature, as indicated by the thermometers, varied between 49.6° and 50° C. One piece was removed after an hour's exposure. Two larvæ isolated from the meat by dissection were alive, but rather sluggish. The other piece was removed after an exposure of 1½ hours. Two larvæ were examined, one of which was dead, the other alive, but rather sluggish. Two guinea pigs were fed with the meat, but neither became infected. Another piece of meat from the same rat was similarly heated for one hour at a temperature of 50.1° to 50.4° C. A larva isolated from the meat after heating was alive and active. Another piece was similarly heated for 1½ hours at 50°. Five larvæ were isolated from the meat and examined. Four were certainly dead, the other inactive, but with protoplasm less changed than that of the others.

EXPERIMENT 26 (August 31, 1914).—Finely chopped meat from a trichinous rat was placed in water in a flask, which was kept 21 hours in an oven maintained at a temperature of 49° to 52° C. The temperature of the water during this time varied from 48.8° to 51.4°. Four larvæ dissected out of the meat after heating were dead. The meat was fed to two rats, both of which remained free from trichinæ. Some finely chopped meat from the same rat was heated 21 hours in a covered Petri dish in the same oven at a temperature of 49° to 52°. Five larvæ dissected out of the meat after heating were dead. Two rats to which the meat was fed remained free from infection.

EXPERIMENT 27 (September 3, 1914).—Finely chopped meat from a trichinous hog was heated in a closed jar in a constant-temperature oven for 19 hours. The temperature of the meat during this time varied between 47.8° and 48.4° C. Twenty-five trichinæ were dissected out of the meat after heating and all found to be dead.

EXPERIMENT 28 (September 8, 1914).—The eviscerated carcass of a trichinous rat was heated 17 hours in an oven at a temperature of 48° to 50° C. On removal from the oven the carcass had a bad odor; the upper surface was dried, the lower still moist. Twenty trichinæ were dissected out of the meat after heating and all found to be dead. Meat from the carcass was then fed to two rats, one of which remained free from trichinæ, while the other was found moderately infected when killed three months after feeding.

EXPERIMENT 29 (September 19, 1914).—Finely chopped meat from a trichinous rat was heated 5 hours in an oven at a temperature of 48° to 49° C. A few trichinæ afterward dissected out of the meat were shrunken, but their protoplasm was bright in appearance. After being soaked in water for 30 minutes some of the larvæ became lively, and 2 days later the remainder of the isolated larvæ kept in water at room temperature had also become active and normal in appearance. Some of the same meat was left in the oven until September 21, and thus exposed for 48 hours to a temperature of 48° to 49° C. It was hard and dry. Trichinæ isolated from the meat by dissection after it had been softened by soaking were very clear, pale, motionless, and apparently dead.

Additional data regarding the effects of the continued action of temperatures below the thermal death point were obtained by the junior writer. In these experiments, which are summarized in tabular form (Table II), the method of procedure was as follows: Meat from trichinous hogs was finely chopped by passing it through a meat chopper several times. A bottle with a capacity of about 200 cc. was half filled with the meat. Through a perforation in the cork a thermometer was inserted into the bottle and the top of the cork then paraffined. The bottle of meat was placed in a constant-temperature oven and the temperatures read on the thermometer in the bottle.

Inasmuch as the meat before being placed in the oven was kept in a refrigerator at a temperature of 8° to 10° C., a considerable period was required to bring its temperature near that of the oven. In nearly all the experiments shown in Table II the meat was in the oven about 2 hours before the first reading of the thermometer, given in the table as the minimum temperature, was made. Between the first and the final reading there was a slight fluctuation of the temperature but nearly always between the limits recorded in the table.

At the end of each experiment a portion of the meat was artificially digested in the usual way and the condition of the larvæ noted. As a control on the microscopic findings in each experiment two rats were fed portions of the meat, being given an average of about 10 gm. each. Unless they died earlier the test animals were killed about a month after feeding. The following table gives the record of 10 experiments:

TABLE II.—*Effects of continued action of temperatures below thermal death point on encysted trichinæ*

| Temperature. | | Period of exposure to given temperatures. | Appearance of larvæ after artificial digestion. | Results of feeding experiments. |
|---|---|---|---|---|
| Minimum. | Maximum. | | | |
| °C. | °C. | Hours. | | |
| 52 | 54 | 4 | Coiled | Negative. |
| 53 | 54.5 | 4½ | Apparently dead | Do. |
| 51 | 56 | 3⅙ | Profoundly disorganized | Do. |
| 52 | 54 | 4 | Showing evidence of having been partially digested | Do. |
| 50 | 52 | 4½ | Uncoiled; evidently dead | Do. |
| 49 | 50 | 5 | Apparently dead | Do. |
| 50 | 50 | 6 | Probably dead | Do. |
| 50 | 50 | 3½ | Uncoiled and pale | Do. |
| 52 | 53 | 6 | .....do | Do. |
| 50 | 54.8 | 4 | .....do | Do. |

From a practical standpoint the results of the experiments on the effects of continuous heating at temperatures below the thermal death point of trichinæ are of comparatively little importance so far as concerns the destruction of the vitality of trichinæ in fresh pork by cooking. Obviously, as compared to cooking at a higher temperature for a short time, there would be no advantage in subjecting meat to a lower temperature, which would require a very great lengthening of the period of heating. If for no other reason, the probable spoiling of the meat would preclude the use of such a method of destroying the vitality of the parasites. In connection with the preparation of certain kinds of cured pork products, however, the fact that heating at low temperatures for considerable periods of time is destructive to the vitality of trichinæ has been put to practical use. In this case there is also another factor which comes into play—namely, the destructive action of salt in hypertonic percentages, which increases greatly as the temperature increases. The question of the destruction of trichinæ in cured pork by heating at low temperatures will be discussed in another paper.

## CONCLUSIONS

The vitality of the larvæ of *Trichinella spiralis* is quickly destroyed by exposure of the parasites to a temperature of 55° C., gradually attained, the result apparently of irreversible coagulation changes in the protoplasm. This temperature may be considered the thermal death point.

Trichina larvæ exposed to temperatures slightly below 55° C. for short periods of time may recover from this exposure; but they die if exposed for longer periods, recovery or death depending apparently upon whether or not beginning coagulation of the protoplasm has proceeded beyond a stage from which a return to normal may occur.

Exposed to temperatures in the neighborhood of 50° C., trichina larvæ die if the application of heat is sufficiently long continued, apparently as a result of exhaustion following excessive activity to which they are stimulated by the heat.

The longevity of trichina larvæ freed from their cysts by artificial digestion and kept at temperatures ranging between limits at which they become quiescent from the effects of heat and cold, respectively, varies inversely with the temperature.

Methods of destroying trichinæ by heating at temperatures below the thermal death point, which may be utilized in connection with the preparation of certain kinds of cured pork products, appear not to be applicable in the case of fresh pork.

Upon the basis of the results of experiments recorded in this paper the Bureau of Animal Industry has selected a temperature of 137° F. (58.33° C.) as the minimum temperature to which pork and products containing pork are required to be heated when cooked in establishments operating under federal meat inspection.[1] This temperature is several degrees above the thermal death point of trichina larvæ, thus providing a certain margin of safety.

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[1] This requirement has reference to the temperature actually reached in the interior of the meat and not merely to that of the water or oven in which it is cooked. It should also be understood that when meat is cooked for purposes of sterilization because of conditions other than trichinosis a higher temperature is necessary than that sufficient to destroy trichinæ.

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